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Volume II.] *October, 1900.* [*No. 1.*

BIOLOGICAL BULLETIN.

A STUDY OF SOME TEXAN PONERINAE.[1]

WILLIAM MORTON WHEELER.

THE observations hitherto published on the habits of the Ponerine subfamily of ants are extremely meager. This gap in our knowledge of ant life must be the more keenly felt by all myrmecologists because the Ponerinae (including the Cerapachyi, a group allied to the Dorylinae, or driver ants) are, to all appearances at least, the most generalized of existing Formicidae. That the Ponerinae have a very primitive morphological and social organization seems to be indicated by the facts, first, that their colonies are composed of a relatively small number of individuals — like the incipient colonies of the more specialized Myrmicine and Formicine ants, and, second, that the polymorphism of the female is still, apparently, in an unstable condition, *i.e.*, the workers often differ little if any from the queens, or females, in form and size, and the two phases are frequently connected by individuals of an intermediate character (ergatoid, or ergatomorphic females). It has also been suspected by Professor Forel and Professor Emery that the breeding habits of the Ponerinae would be found to be of a primitive nature.

The common occurrence of three beautiful species of Ponerinae in the environs of Austin, Texas, has induced me to undertake the following study of their habits. The ants were observed in a state of nature or kept in jars of earth or in artificial nests of the Lubbock and of the Janet pattern. In collecting material for these nests, I have been considerably aided by two of my students, Mr. A. L. Melander and Mr. C. T. Brues. Mr. Brues also aided me in drawing some of the figures that accompany this paper.

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[1] *Contributions from the Zoölogical Laboratory of the University of Texas.* Director, W. M. Wheeler. No. 6.

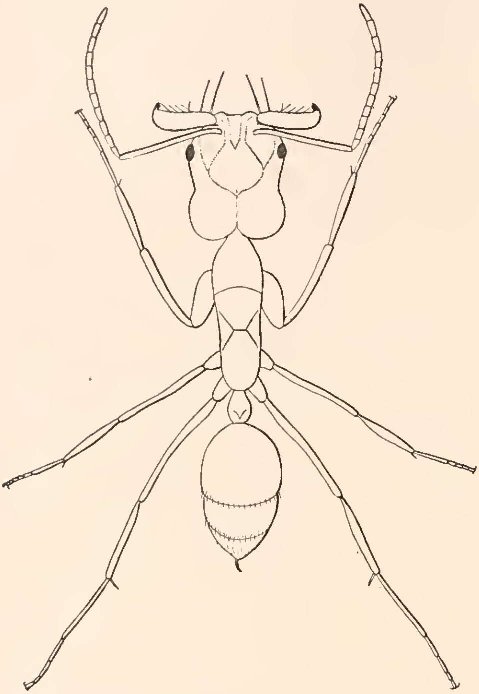

FIG. 1. — *Odontomachus haematodes* Linn. Worker.

The three species of Ponerinae which I have studied are: *Odontomachus haematodes* L.; *Pachycondyla harpax* Fabr. (= *Ponera amplinoda* Buckley); and *Leptogenys elongata* Buckley.[1] To European myrmecologists both *O. haematodes* and *P. harpax* are well-known species. They have a wide geographical distribution, occurring from Texas through Mexico, Central America and Brazil to Bolivia and Paraguay. *O. haematodes* is also recorded from Georgia, Florida, and the Antilles.[2] In the vicinity of Austin, *P. harpax* and *L. elongata* are found under stones or logs, on moderately dry hill-slopes, often in the shade of trees or bushes, more rarely in the open fields. In these same localities *O. haematodes* does not occur. The few nests which I have seen belonging to this species were under stones lying on a dry sandy loam in a different part of Travis County. The exact distribution of these forms must be left for future determination.

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[1] Specimens of the last species were kindly identified for me by Mr. Pergande as *Lobopelta elongata* Buck.? The query seems to be due to Buckley's wretched description of his *Ponera elongata*. Since Lobopelta is treated as a subgenus of Leptogenys by Forel, and since the species is very common in Texas, and therefore, in all probability, the one described by Buckley, I have changed the generic name and omitted the query. Should doubts arise, my figures (Fig. 4) will, I trust, enable any future observer to recognize the species.

[2] See Emery, "Beiträge zur Kenntniss der nordamerikanischen Ameisenfauna," *Zool. Jahrb.*, Abth. f. Systematik., Bd. viii, pp. 268, 269; Patton, *Amer. Nat.*, July, 1894, pp. 618, 619; Forel, Biologia Centrali-Americana, Hymenoptera, vol. iii, April, 1899, p. 21.

It is not an easy matter to ascertain the exact number of individuals in any colony of the three species of Ponerinae. When the stone or log that serves as a roof to the nest is lifted, some of the ants always manage to escape, either into the surrounding vegetation or into their burrows. This is especially true of *L. elongata* and *P. harpax*, both of which, when excited, are very rapid in their movements. As a rough estimate it may be said that the nests of *L. elongata* contain from 10 to 50, those of *P. harpax* from 15 to 100, of *O. haematodes* from 100 to 200 individuals.[1]

The nests of the three Ponerinae agree in being of a very primitive structure. They consist of a few simple and irregular burrows, or galleries, some of which run along the surface of the soil immediately beneath the stone or log, while others extend down into the soil obliquely or vertically to a depth of 8 or 10 inches. These burrows may anastomose, but they are not widened at certain points to form chambers, as in the nests of the more specialized ants (Atta, Pogonomyrmex, Camponotus, etc.). Even in artificial nests of the Lubbock pattern the Ponerinae dig only anastomosing galleries scarcely more than a centimeter in diameter.

Owing to the interest which attaches to the sexual phases of the Ponerinae, considerable effort was made to secure both the winged and apterous forms of the Texan species. Of *O. haematodes* only workers were seen, but as the full series of phases of this species — including the winged male, winged female, and the apterous ergatoid female, as well as the worker

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[1] In tropical America (*teste* Forel, *loc. cit.*, *passim*) *P. harpax*, race *Montezumia*, Smith, *O. haematodes*, and the species of Leptogenys make their nests also in rotten wood, and the first species is said to nest also in dry, hollow stems. The colonies of *O. haematodes* are probably more numerous in individuals in the tropics than at Austin.

— have been made known through the labors of European myrmecologists, I fixed my attention on the other species. I append a brief description of these species before recording their habits.

P. harpax (Figs. 2 and 3) is a large, rather robust, subopaque, black ant, which carries its body close to the ground. It has a peculiar habit of folding its antennae and of peeping out of holes and crevices in the soil like a rat. In nearly all the nests that were opened only wingless individuals like the one represented in Fig. 3 were found. Except for a slight variation in size they were all alike externally, and I at first regarded them all as workers; but on dissecting several of these ants during the breeding season (from the 1st of March to the end of May) some of them were found to contain mature ova, while others had abortive ovaries. In a nest consisting of twenty-nine individuals captured May 27, dissection revealed the presence of mature eggs in thirteen, and one-third to one-half grown eggs in four of the ants. The ants were also caring for several

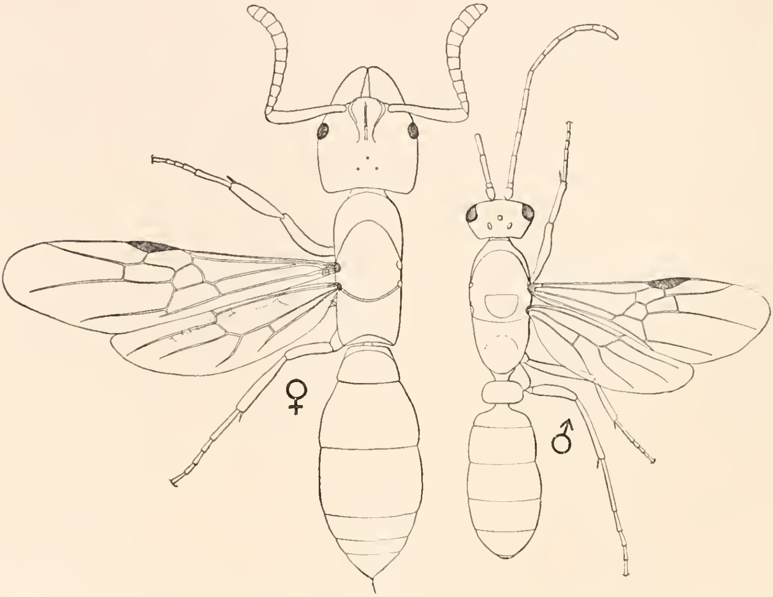

FIG. 2. — *Pachycondyla harpax* Fabr. Winged male and female.

packets of eggs which they had laid. Like the pigeons among birds, *P. harpax* lays only two eggs at a time. I infer this from the fact that only two eggs are found to be mature in the ovaries. Since, in addition to these facts, wingless individuals actually laid eggs in my artificial nests, I feel certain that forms externally indistinguishable from the workers commonly function as females. These are, therefore, ergatoid or ergatomorphic females. There is not even a slight difference in size to separate the two forms externally, since some of the ergatoid females are smaller than some of the workers. There are, however, in certain nests of *P. harpax* winged females of a less

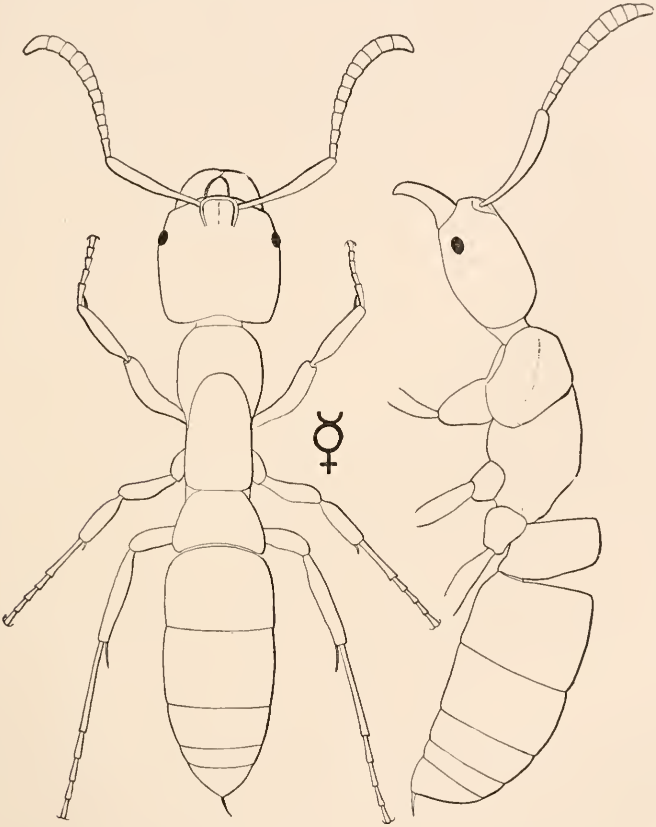

Fig. 3. — *Pachycondyla harpax* Fabr. Worker, dorsal and lateral aspect.

worker-like aspect. These were seen only twice — once in a large nest found under a log on March 12, and again in a nest discovered in a very different locality by Mr. Brues, March 31. In both nests there were many of these winged females resembling Fig. 2, ♀. They had small but distinct ocelli. Several of them had lost some of their wings, while a slight touch caused others to lose these appendages. Before finding these winged females, I had found the winged males as early as March 3 in a small nest containing ergatoid females and workers. There were two of these males clinging to the under surface of the stone that covered the surface galleries of the nest. They differ so much from the apterous females and workers, that, if taken on the wing, they would scarcely be regarded as ants (*cf.* Fig. 2, ♂, and Fig. 3). They are much shorter and more slender than the workers, and their long, slender, 13-jointed antennae are not geniculate, but straight, with the basal and second joints short and the remaining joints slender and subequal. The eyes and ocelli are very large and prominent. These and other equally pronounced structural peculiarities in the thorax and abdomen are shown in Fig. 2, ♂. The small mouth-parts, the hypopygium, trochanters, tibiae, and tarsi are honey-yellow; the venter and sides of the abdomen are piceous.[1] On the 31st of March I found four nests containing winged males. Three of these nests each contained three males, the remaining nest contained only two.

L. elongata is smaller and far more slender than *P. harpax*, with long delicate legs and antennae. The specific name is very applicable not only to the adult ant but also to the egg, larva, and pupa. The worker (Fig. 4, ☿) is of a rich claret red color, becoming yellow towards the tip of the abdomen. In form, color, and movement this ant is certainly the embodiment of elegance. The workers which were dissected — some thirty specimens from different nests — contained only minute and abortive ova, and for some time I failed to find the queen.

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[1] To my knowledge no description has hitherto been published of the male of the typical *P. harpax*. I infer, however, from Forel's contribution (*loc. cit.*, p. 12) that Smith has described (*Cat. Hym.*, vol. vi, p. 108, 1858) the winged male of his *P. montezumia*, a form which Forel regards as merely a Mexican race of *P. harpax*.

Finally I noticed in each nest a single ant with a somewhat larger abdomen than that of the workers, but, like them, without any traces of wings. Dissection of three such individuals disclosed mature eggs — only a single pair in each insect, as in Pachycondyla. There could be no doubt that I had found the hitherto unknown female of Leptogenys.[1] They have no ocelli,

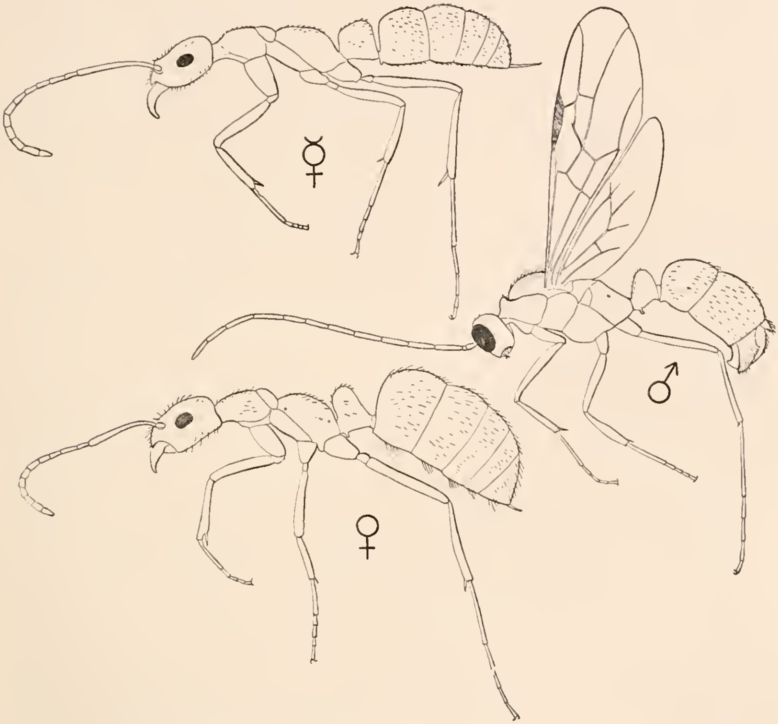

Fig. 4. — *Leptogenys* (*Lobopelta*) *elongata* Buck. Worker, winged male, and apterous female.

but the shape of the node and of the abdomen serves to distinguish them at once from the workers (see Fig. 4, ♀). They are certainly ergatoid females, but as such they differ from the ergatoid females of *P. harpax* in several respects: First, they resemble the workers less closely; second, they occur singly in the nests; and, third, they have the status of true queens, because they are the only known egg-laying individuals of the species. The winged, light-yellow male of *L. elongata* was determined on circumstantial evidence, as it could not be found in the natural nests, nor bred from the cocoons in the artificial nests. What seemed to be the males (Fig. 4, ♂) were found during the autumn and spring flying about the lights in the houses. They are somewhat smaller than the workers, have very large eyes and ocelli, and the node is shaped like that of the female. When placed in the artificial nests of *L. elongata* they are not only not molested, but move about among the workers as if they had always belonged among them. To one who has witnessed the hostility of these ants to any ant of a different species placed in their nest, this amicable reception amounts to a demonstration of specific identity.

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[1] Thus Emery's supposition (Emery, C., "Zur Biologie der Ameisen," *Biol. Centralbl.*, Bd. xi, p. 174, 1891), that the females of this genus are apterous, is proved to be true of at least one species.

During the course of my observations I have had frequent opportunity to observe the conduct of all three species of Ponerinae towards ants of the same or of different species introduced into their nests. They are always hostile to ants of a different species, but the aggressive nature of this hostility varies according to circumstances. If one or a few strange ants be placed in a nest of Ponerinae, they will be at once attacked and killed by concerted action, but if a single Ponerine be placed in the nest of another species — say in a nest of the agricultural ant (*Pogonomyrmex barbatus*) — it will if attacked defend itself valiantly with its mandibles and sting, but it will prefer to seek refuge in some corner of the nest and remain concealed. Single specimens of *P. harpax*, introduced at different times into the nest of *P. barbatus*, managed to evade the pugnacious agriculturals and to remain alive for days. Whenever they left their concealment, however, they would be attacked by a swarm of agriculturals, but the Ponerinae were so supple and fierce that they always managed to throw off their assailants and to conceal themselves. Other investigators, like Forel and Wasmann, have called attention to this difference in the pugnacity of ants according to whether they find themselves alone or backed by numbers of their own species.

The species of Ponerinae differ in their reception of ants of the same species from other nests. If two nests of workers of *P. harpax* (including ergatoid females) be thrown together into the same jar, the ants of different nests at once begin to struggle with one another. One ant will grasp a stranger by the head with her mandibles and smite her with rapidly vibrating antennae; or the two foes will interlock mandibles and pull in opposite directions till one gives up and runs away, only to begin the same performance with some other stranger. This peculiar tugging goes on between the ants of different nests for hours, or even for three or four days. Then, without any mutilations or deaths as a result of the struggle, the ants abandon all this hostile play, and thenceforth form a single peaceable community.

When two nests of workers of *L. elongata* are put together, the struggle which ensues presents a slightly different aspect. An ant from one nest will grasp a stranger by an antenna or by one of her fore feet and, while tugging, smite her opponent with very rapidly vibrating antennae. In this species there is no interlocking of mandibles. After several days of tugging and struggling the same peaceable conditions supervene as in the case of *P. harpax*.

Workers of *O. haematodes*, belonging to different nests, never display the slightest animosity towards each other, so far as I have been able to observe: in a strange nest they are treated exactly as if they had always belonged there. An exception to this conduct of *O. haematodes* in the case of friends deprived of their antennae will be mentioned below.

A few observations were made on the conduct of nests of workers towards sexual individuals from other nests. One of the two males of *P. harpax*, taken March 3, was placed in a strange nest of ten workers (including ergatoid females). The male, after walking about on the earth for a short time, found his way into one of the galleries in which six of the wingless individuals were digging. On perceiving the male they at once stopped their work and began licking the winged stranger with signs of great agitation and affection. At the same time they carefully massaged all his limbs and segments with their mandibles. During this effusion the attitude of the male was most peculiar. He lay on his back in what may be described as a pupal attitude, with his legs drawn up against his body, and his long filiform antennae folded against the mid-ventral surface of his thorax and abdomen. This amusing scene was still in progress when I had to quit my observations half an hour later. The next morning I saw one of the workers (or an ergatoid female?) carry the male in her mandibles a distance of four inches and disappear with him into one of the galleries. During transport the male preserved the motionless pupal attitude. This treatment of a strange male contrasts strikingly with the treatment received by the male which was allowed to remain with the workers and ergatoids of his own colony. After eight days of captivity this male was killed and devoured by one of the wingless individuals. When I first observed this Amazon, she had already consumed the small head of her victim and was hanging from the roof of a gallery, twirling the torso about with her feet and rasping away the thoracic muscles with her maxillae. Was this act prompted by hunger? or had the male fulfilled his mission in this nest already provided with several packets of eggs? I have already mentioned the fact that the male of *L. elongata* was received by a strange colony of workers like an old acquaintance. The absence of any display of affection in this case may have been due to the fact that there were no females in the nest. Two strange females introduced into this same nest at different times were at once surrounded and attacked by the workers, and although the colony was without a queen, they were either maimed or killed during the course of the day.

Many of the habits of the Ponerinae closely resemble those of other ants. All the habits relating to the cleanliness of the individual ants and of the nests are the same as those of the Myrmicinae and Formicinae. They clean themselves and one another with great care. The males spend much of their time drawing their delicate antennae through the strigils on the fore feet. The nests of all the species are kept scrupulously clean — all refuse, inedible fragments of their insect prey, empty cocoons, dead sister ants, etc., are carefully deposited in one of the corners of the nest exposed to the light and as far as possible from the chambers in which the young are reared.

In digging a gallery *P. harpax* first uses its feet and powerful mandibles in removing the earth, after the manner of the digger-wasps, but as soon as the gallery is sunk to a certain depth, only the jaws are used in removing the lumps of earth.

Emery's observations[1] show that the Ponerinae have the power of stridulating. He has even been able to produce the sound in preserved specimens of certain American species, including species of Pachycondyla. Although the ants observed by me occasionally moved their abdomens as if stridulating, no sound could be detected.

The sting of the three species of Ponerinae, although of formidable length, does not inflict severe pain, at least when compared with the sting of the agricultural ant (*Pogonomyrmex barbatus* Sm.). The pain may be acute for ten or fifteen minutes, but then disappears, often without even reddening the skin ; whereas the sting of Pogonomyrmex produces a throbbing pain which endures for hours and is sometimes accompanied by a sensation of sickness.

The Ponerinae do not seem to feed one another, like the specialized ants. In captivity *P. harpax* would eat the yolk of egg or even sugar, but it would not eat termites. *L. elongata* devoured termites and small caterpillars with avidity, but would not eat flies. *O. haematodes* is more omnivorous ; besides caterpillars, house-flies, beetles, and small Hemiptera, it will eat sugar, bread, cake, etc.

Pachycondyla and Leptogenys are like other ants in their methods of killing their prey or in feeding upon it, but Odontomachus is most exceptional in these particulars. Its whole life, apart from the care of its young, appears to center in its peculiarly constructed mouth. The long linear mandibles with hook-shaped dentate tips are inserted close together. They are usually carried wide open, as represented in Fig. 1, while the ant is moving about in search of food or while it is feeding. The cutting edges of the mandibles are furnished with some sensory hairs, two of which, nearly as long as the mandibles, are inserted near the base and point directly forward when the mouth-parts are in the position represented in Fig. 1. The slender antennae, too, are carried in a peculiar position, their tips being directed inwards. The touching of a living insect or of any unfamiliar object with these incurved tips calls forth a peculiar response, which seems to be largely of a reflex nature. The ant darts forward and suddenly closes its mandibles with a very audible click. The signal for closing the mandibles seems to be given the moment the long sense-hairs touch the object. The mandibles are brought together with such force that if they strike a solid object the ant is thrown backwards — often to a distance of three or four inches — occasionally even to a distance of ten or twelve inches. The ant alights on its feet, like a cat, and again advances to repeat the act. This remarkable clicking and leaping habit is called into play on every occasion, and its study discloses some interesting facts, as the following jottings from my notebook will show:

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[1] Emery, C., "Zirpende und springende Ameisen," *Biol. Centralbl.* Bd. xiii, pp. 189–190. 1893.

May 12. Placed a living house-fly in the Odontomachus nest. Its movements at once attracted several ants, which began snapping at it like a pack of angry dogs. With each snap a leg or wing was severed and often thrown to a distance of 2 or 3 inches. In less than a minute all the limbs had been shorn from the trunk. The fly was then seized and decapitated. Next the following living ants were placed in the nest in succession: *Eciton sumichrasti*, *Pogonomyrmex barbatus*, and *Leptogenys elongata*. The first and last were reduced to torsos as rapidly as the fly; the harder legs and antennae of the Pogonomyrmex offered greater resistance, but not for long. The stings and strong mandibles of these various ants were no protection against the singular method of attack adopted by the Odontomachus. A smooth green caterpillar was next introduced into the nest. It was at once surrounded and attacked by a dozen ants. At first the ants retreated from the writhing larva after each snapping of the mandibles, but soon they grew bolder and, retaining their hold, drove their stings through its velvety skin. One ant with a dexterous movement closed its mandibles in the caterpillar's brain. In a few moments the green blood of the victim was oozing from a score of wounds.

May 25. *O. haematodes* reacts to lifeless objects just as it does towards living insects. If the points of a pair of tweezers be held in the nest and rapidly opened and closed, they at once become the center of a swarm of snapping and leaping ants. A small glass vial dropped into the nest at once begins to tinkle under the blows of the alternately advancing and retreating emmets. A lump of sugar is attacked in the same manner for some moments, till the ants learn that it is edible, when they settle down on it and lap its crystals with avidity. Even soft viscid or liquid substances, like the yolk of egg, poured on the floor of the nest, at once release the same peculiar response. As soon as the ants perceive it with the contact-odor sense of their antennae, they begin snapping at it as if it were a deadly foe. In this case the tips of their mandibles are smeared with the yolk. This causes them to feel some discomfort apparently, and they wipe them on the hard floor of the nest, much as a bird would wipe its beak in similar circumstances. It is only after several repetitions of this performance that they approach the yolk without closing the mandibles. Then they begin to eat it, and some hours later they begin to cover it up with little pellets of earth, for *O. haematodes*, as well as *L. elongata* and *P. harpax*, share with the Myrmicine and Formicine (and I may add, also, the Doryline ants!) the habit of burying offensive substances.

Observers[1] have called attention to the fact that ants deprived of their antennae are in some cases attacked and killed by ants of the same nest. A few experiments were tried for the sake of testing these observations on Odontomachus. One of these ants, deprived of both antennae, was replaced in the nest from which it had just been taken. It was at once fiercely attacked by several ants as if it had been a strange insect. It stopped, as if dazed, unable to meet its sister workers with the customary antennal greeting. The ants, however, soon perceived their error, and a few hours later one of them was licking and fondling the mutilated ant. By the next day the mutilated ant was dead, probably as a result of the extirpation of its antennae. Two other experiments terminated in the same way.

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[1] Forel, Fourmis de la Suisse, p. 119, *et al.*; Wasmann, Die zusammengesetzten Nester und gemischten Kolonien der Ameisen, pp. 151, 152, Münster i. W., 1891.

Two inferences may, I believe, be drawn from these observations on the snapping and jumping habits of Odontomachus: First, it seems evident that these are reflex actions; second, that they can be inhibited by the will of the insect. This latter conclusion is supported by the experiment with the sugar and the yolk. It also shows that these ants learn by experience, and that they possess some form of memory.

The peculiar snapping and leaping habits of *O. haematodes* have been remarked by a few other observers. Emery has published a brief note on this subject,[1] and Forel [2] says of this insect that "en Colombie on l'appelle 'Fourmi tac', à cause du bruit qu'il fait en refermant brusquement ses mandibules. Par le même mouvement il ressaute en arrière lorsqu'il les referme contre un objet, ce qui lui a fait attribuer à tort la propriété de sauter." I cannot accept this last statement, which seems to be a contradiction in terms. The ant makes use of its saltatory powers for purposes of escape, as any one who tries to capture a colony of these ants may readily observe. That it leaps backward instead of forward, like other insects, is due to its using a most unusual organ for leaping, for the mandibles, which, as in other ants, are used for digging and transporting the soil, carrying eggs and larvae, and for killing and cutting up prey, have acquired an additional function as saltatory organs in *O. haematodes*.

The breeding habits and the characteristics of the eggs and larvae of the Ponerinae exhibit striking deviations from those of other ants. I have not seen the eggs of Odontomachus, but throughout the month of May I have often happened on the eggs of Pachycondyla and Leptogenys. These are white and of a slender, oblong shape (Fig. 8, *a*), somewhat smaller in the latter than in the former genus. They differ in shape from the eggs of species of Eciton, Camponotus, Formica, Pogonomyrmex, Solenopsis, and Tapinoma; for the ants of these genera, representing several subfamilies, agree in having elliptical and much less slender eggs than the Ponerinae. The Ponerinae also keep their eggs in more regular packets, the long axes of the different eggs being placed parallel with one another. I have not been able to determine the length of embryonic development with accuracy. It seems to be much protracted, as in other ants. Some eggs of *P. harpax* in an artificial nest had not hatched at the end of five weeks.

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[1] *Biol. Centralbl.* Bd. xiii, pp. 189–190. 1893.

[2] *Loc. cit.*, p. 20.

The larvae of the Ponerinae differ from those of all other ants in several particulars, first made known by Emery.[1] Emery describes and figures the larvae of *Ponera stigma* Fab. (New Guinea), *P. caffraria* F. Sm. (Cape Colony), *Diacamma rugosum-geometricum* F. Sm. (Celebes), and *Odontomachus haematodes* L. (Cayenne). All of these larvae agree in having the mandibles powerfully developed for ant-larvae, the anterior portion of the body long and slender and folded over the abdominal portion, and in being covered with rows of peculiar tubercles beset with more or less prominent bristles.

The larvae of Pachycondyla and Leptogenys are here figured for the first time. I have seen fit to figure also the Odontomachus larvae because my material was evidently in fresher condition than that depicted by Professor Emery. The larvae of the three genera may be arranged in a series beginning with Odontomachus and terminating with Pachycondyla.

The young larva of *O. haematodes* is represented in Fig. 5, *a*, which shows the arrangement and character of the bristly tubercules and the neck-like anterior portion, consisting of the head, the three thoracic and the first two abdominal segments. The remaining eight abdominal segments are much enlarged and flattened ventrally. The larva is kept on its back, and the neck-like anterior portion rests against the flattened ventral surface. The shape of the tubercles, each of which is tipped with a rigid bristle and encircled with bristles, is shown in Fig. 5, *b*. The larva was about to moult, so that the tubercle of the succeeding cuticle is seen shining through the old one. The adult larva is shown in Fig. 6, *a*. Compared with that of the young larva, its head is very small in proportion to the body.

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[1] "Intorno alle Larve di Alcune Formiche," *Mem. della Accad. delle Scienze dell Istituto di Bologna*, 7. Maggio, 1899, 2. Tavole.

This seems to be the universal rule in ant larvae. The head in dorsal view is represented enlarged in Fig. 6, *b*. The powerful dentate mandibles lie just below the outer edges of the bilobed labrum ; still lower and projecting forward lies the labium, bearing on its tip the opening of the spinning gland (to be used in weaving the cocoon), and on either side two peg-shaped tactile (?) organs. Similar but somewhat larger organs are seen on the edges of the maxillae, which protrude on either side below the mandibles. The ten tracheal stigmata, beginning on the mesothoracic and terminating on the eighth abdominal segment, are clearly shown in Fig. 6, *a*. The bristly tubercles are essentially the same in structure as those of the younger larva, but they are relatively shorter and smaller. (*Cf.* Fig. 5, *b*, and Fig. 6, *c*.)

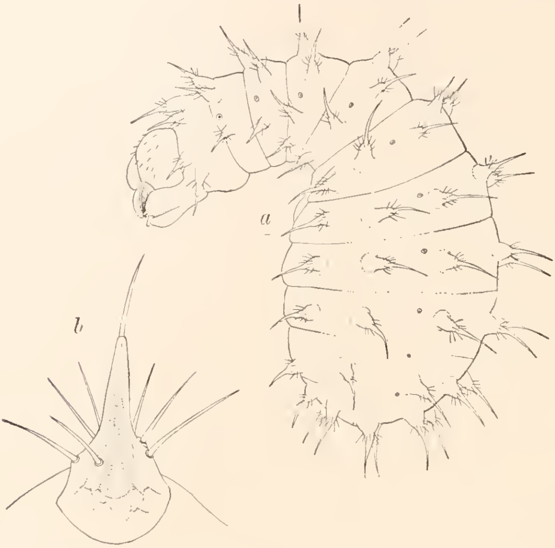

Fig. 5. — *Odontomachus haematodes* Linn. *a*, young larva ; *b*, tubercle of the same.

The larvae of Leptogenys (Fig. 7) are remarkably slender and scarcely flattened on the ventral surface. In the young larvae (Fig. 7, *a*) the tubercles are distinctly curved and pointed, without apical bristle, and with only a few rather short bristles encircling the base (Fig. 7, *d*). In the adult larvae (Fig. 7, *b* and *e*) the tubercles are larger and shorter, with blunt or acuminate apex and with relatively longer and more numerous basal bristles. The head of the adult larva (Fig. 7, *c*) is remarkable for its length and the narrowness of the labrum, which is nearly as long as the slender mandibles and provided with a median tooth at its tip.

The larvae of Pachycondyla (Fig. 8) are neither as slender as those of Leptogenys nor as robust as those of Odontomachus.

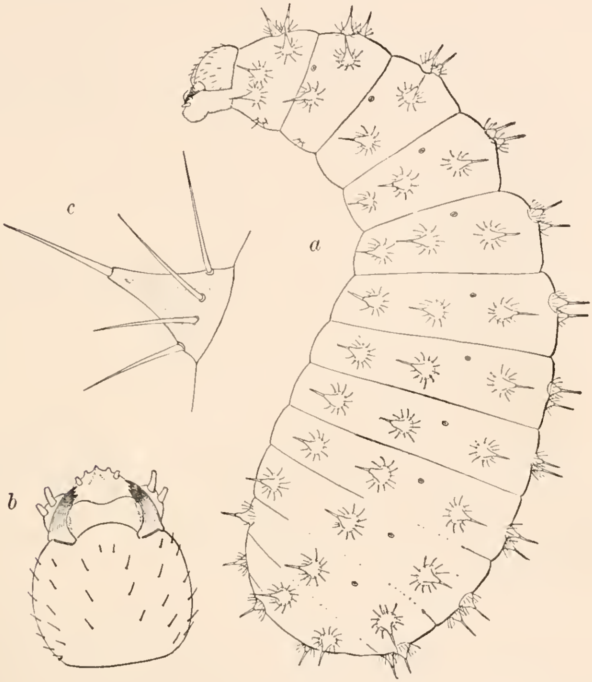

Fig. 6.—*Odontomachus haematodes* Linn. *a*, adult larva; *b*, head of same (dorsal aspect); *c*, tubercle.

The ventral surface of the abdomen is distinctly flattened. The head (Fig. 8, *e*) resembles that of Odontomachus, especially in the shape of the labrum and mouth-parts. There is a striking difference between the tubercles of the very young and the adult larva. In the former (Fig. 8, *b*, *c*) the tubercles are nearly or quite straight, and somewhat longer and more pointed than those of Leptogenys. They lack the terminal bristle. The bristles about the base are somewhat irregular in their insertion.

In the adult larva (Fig. 8, *d*) the tubercles are reduced to large more or less flattened bosses, encircled with a regular row of numerous, rather long bristles. In the stages between those figured the gradual flattening of the juvenile spine-like tubercles can be traced through the successive moults.

In this series of larval forms, Odontomachus seems to represent the most primitive condition. Here both young and old larvae have pointed, bristle-tipped tubercles, and there is little difference between the tubercles of the young and adult. In Leptogenys and Pachycondyla the apical bristle is absent, but in both genera the young larvae have pointed tubercles. In the adult larva of the former genus there is a perceptible blunting of the tubercles, while in the adult larva of the latter the tubercles have nearly subsided.[1]

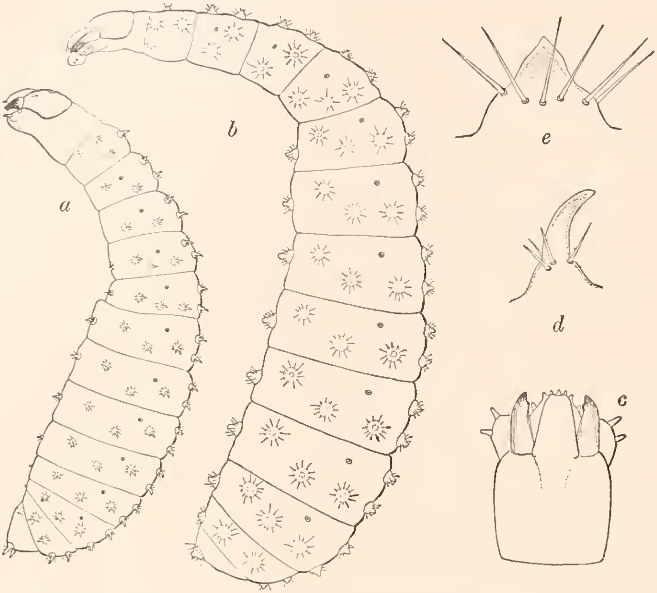

FIG. 7. — *Leptogenys* (*Lobopelta*) *elongata* Buck. *a*, young; *b*, adult larva; *c*, head of adult larva (dorsal aspect); *d*, tubercle of young; *e*, tubercle of adult larva.

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[1] Emery's observations on *Ponera stigma*, *P. caffraria*, and *Diacamma geometricum*, seem to indicate conditions the reverse of those which I have described. Of the former species he says (*loc. cit.*, p. 4); "Nello stado piu giovane, si vedono solo deboli accenni dei tubercoli cutanei; ritengo che questo stado debba corrispondere alle larve di prima schiusa e che lo stado seguente, di poco piu grande, sia quello

The bristly tubercles of the larvae of the Ponerinae are so prominent as readily to suggest the question of their function. Prof. L. Biró, who made some observations on the larvae of *P. stigma*, which he sent to Professor Emery, believes that the pointed tubercles are organs of defense. He saw these larvae when disturbed by some termites move their long necks back and forth with sufficient force to drive away the intruders.[1]

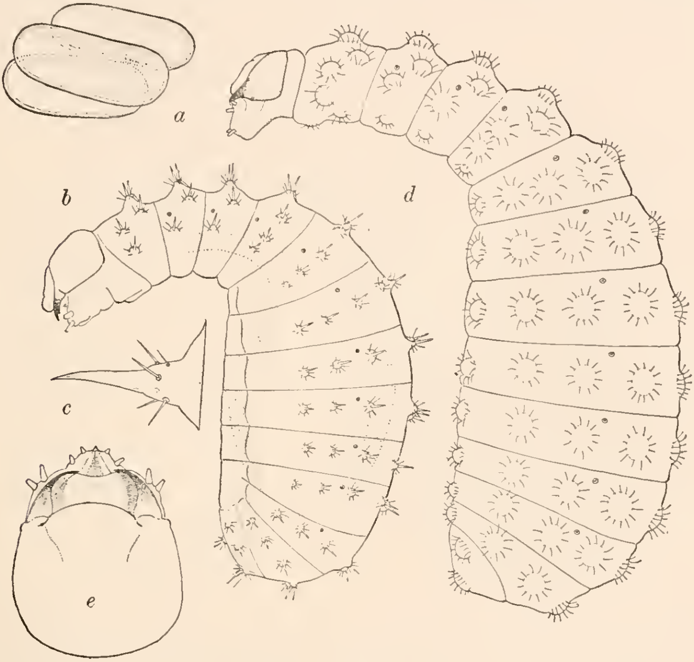

FIG. 8.—*Pachycondyla harpax* Fabr. *a*, eggs; *b*, young larva; *c*, tubercle of the same; *d*, adult larva; *e*, head of the same.

che segue la prima muta; questi si fanno successivamente piu numerosi e sporgenti, a misura che la larva cresce." In the larvae of Diacamma a very different condition is described: "Sopra ciascuno (segmento) di essi si trova un serie transversale, irregolare di tubercoli conici, ineguali che, nelle larve piu sviluppate, portano da uno a quattro peli. Nelle piccole larve, i tubercoli sono piccoli, subcilindrici e senza peli; negli stadi intermedii passano per una forma acuminata con pochi peli."

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[1] Professor Emery (*loc. cit.*, p. 4) quotes from Professor Biró's letter: "Nelle gallerie del nido scavato nel legno putrido, si trovavano le larve dal lungo collo, coperte di spini singolari: abbandonate dai loro vigliacchi custodi, quelle larve sapevano difendersi da se; quando qualche termite (il nido di queste trovavasi nello stesso legno) si avvicinava ad una di esse, questa batteva innanzi e indietro col suo collo di cigno e tosto veniva lasciata in pace."

Biró's observations may be true of *P. stigma* without being applicable to the three forms of Ponerinae which I have observed. In artificial nests I have seen the neck movements of the larvae, but they were often executed when the larva was undisturbed, except perhaps by the pangs of hunger, and they were not always made when termites or other insects were running about and over them. Moreover, we should expect to find the tubercles more highly developed on the neck than on the body, if they are really used as Biró suggests. I believe that while they may be organs of passive defense, like the somewhat similar tubercles and spines of certain caterpillars, they also fulfill other functions; they would seem to facilitate the carrying of the larvae either singly — when full-grown — or in batches — when young — by the worker ants. In the last instance they would represent a peculiar form of the "poils d'accrochage" carefully studied by Janet.[1] Janet finds that the young larvae of the more specialized ants are covered with

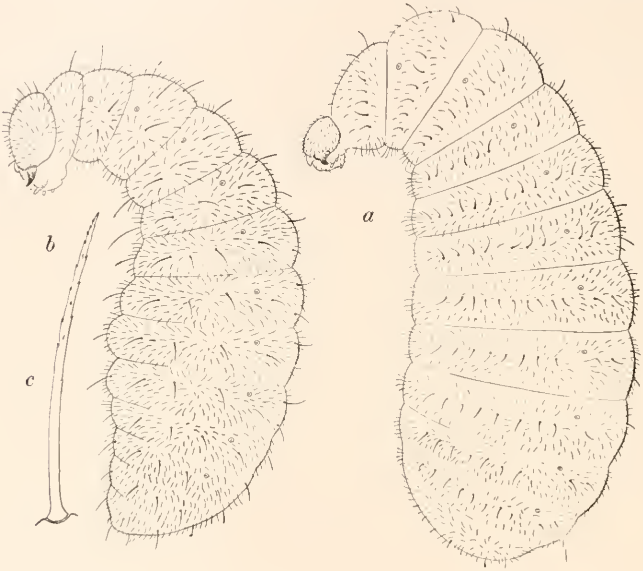

FIG. 9. — *Pogonomyrmex barbatus* Smith. *a*, nearly adult larva; *b*, young larva; *c*, serrated bristle of the same.

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[1] Les Fourmis. Conférence faite le 28 Février, 1896. Paris, 1896.

hooked bristles, which cause them to adhere together in packets and thus facilitate their transportation by the workers. The appearance of these peculiar hairs in the young and half-grown larvae of one of our common Texas ants, *Solenopsis geminata* Fabr., is shown in Fig. 10. The very young larvae have only simple bifurcated hairs, but when half-grown they have on the dorsal surface of several of the segments, besides a much greater number of these simple bifurcated hairs, several rows of long and peculiarly contorted bristles, terminating in short bifurcations. Still another modification of the "poils d'accrochages" is seen in *Pogonomyrmex barbatus* (Fig. 9), the young larvae of which have the longer bristles serrate on the apical half, so that they remind one of the hairs of certain mammals. All of these modifications — the bristly tubercles of the Ponerinae, the simple and contorted bifurcated bristles of Solenopsis, the serrate bristles of Pogonomyrmex, and possibly also the fascicles of uncinate hairs described and figured by Emery (*loc. cit.*) for the larva of *Sima natalensis* F. Sm. — seem to subserve the same purpose — a most interesting example of independent

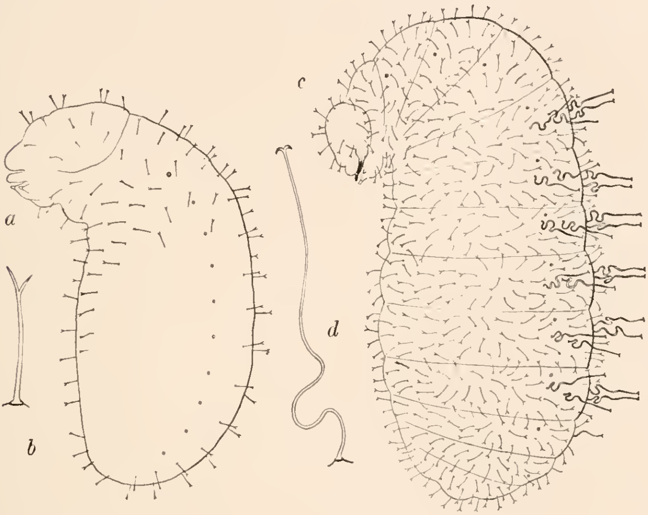

Fig. 10. — *Solenopsis geminata* Fabr. *a*, very young larva; *b*, furcate bristle of same; *c*, half-grown larva; *d*, contorted furcate bristle of same.

lines of development terminating in organs of different structure but identical function.

The pupae of Odontomachus, Leptogenys, and Pachycondyla are enclosed in elliptical brown cocoons, like the pupae of many species of Formica and Camponotus. The pupa of *L. elongata* is remarkable on account of its very slender shape, a peculiarity not confined to the pupa, but, as we have seen, extending also to the egg, larva, and imago.

We come now to a consideration of the breeding habits of the Ponerinae. The little that has been made known concerning their habits has led European myrmecologists to believe that the philoprogenitive instincts of these ants must be less highly developed than those of the Myrmicinae and Formicinae. Thus, according to the above-quoted note of Professor Biró, when the nest of *Ponera stigma* is disturbed the ants flee and the larvae are "abbandonate dai loro vigliacchi custodi." And Professor Forel has made what appears to be a somewhat similar observation on our American *Ponera coarctata* Fabr. in North Carolina[1]: "La *Ponera coarctata* americaine est très commune dans les troncs pourris et sous les pierres. J'ai fait chez elle une observation qu'il est bien difficile de faire en Europe; mais ici elle est tout à fait constante. Lorsqu'on découvre un nid de Ponera dans un tronc pourri, on voit leurs cocons jaunes assemblés dans un coin, mais absolument abandonnés des ☿ qui n'essaient pas de les sauver, ni de les recueiller. Par contre, elles prennent le plus grand soin des larves qu'elles emportent et cachent. Je soupçonne que chez ces fourmis, moins sociales que les autres, les nymphes sortent seules de leurs cocons, sans avoir besoin de l'aide des ☿."

These observations relate to species of Ponera and are at variance with the conclusions which I have reached from a study of three other genera of Ponerinae. In many of the nests which I have examined the total number of the eggs, larvae and pupae, could scarcely be greater than one and one-half to twice the number of the ants. This fact, together with what has been said of the small number of eggs laid at one time by a single female, shows very clearly that the Ponerinae are not nearly so prolific as the species of Camponotus, Formica, Pogonomyrmex, Pheidole, Tapinoma, Eciton, etc. Indeed, the small number of ants in the nests of the Ponerinae is probably the direct result of this limited productivity. If this is the case, it does not seem probable that these ants would be more careless of their progeny than the very prolific specialized ants. On the contrary, we should expect them to extend even greater protection to their offspring. This my observations show to be the case; at any rate, *P. harpax*, *L. elongata*, and *O. haematodes* are in nowise inferior to the Myrmicinae and Formicinae in this respect. The slightest disturbance of the natural or artificial nests of these ants causes them at once to seize their eggs, larvae, and cocoons, and to make for their galleries. Occasionally some of the ants escape without anything, but if they are watched for a few moments, they will be seen returning, often in the very face of danger, to carry off more of their young. They are, it is true, most careful of their eggs, somewhat less careful of the larvae, and least careful of their cocoons; but these distinctions are not always apparent and can only be affirmed as the result of many observations. When the colony is agitated, it is probably most easy for the ants to seize and remove the small packets of eggs and the younger larvae, and least easy to carry off the larger larvae and the awkward cocoons. Dead pupae are often collected in one part of the nest and are there allowed to lie unheeded. I am inclined to think that Professor Forel may have seen such abandoned pupae in the nests of *P. coarctata*.

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[1] *Ann. de la Soc. Entomol. de Belgique.* Tome 43, p. 443. 1899.

The strong development of the mandibles of the Ponerine larva as compared with those of other ants led Emery remotely to surmise the method which the Ponerinae employ in feeding their young.[1] But no myrmecologist could have predicted the remarkable and un-ant-like procedure which I have been able to observe in the three Texan species.

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[1] *Loc. cit.*, pp. 8, 9. "Sembrami pertanto che lo sviluppo notevole della bocca e particolarmente delle mandibole, nelle larve delle Ponerinae e dell' Acanthostichus inducano a qualche supposizione relativamente alla biologia di queste formiche. Le larve delle specie europee che finora furono osservate vengono alimentate col contenuto dell' ingluvie delle operaie che queste regurgitano sulla bocca delle loro larve, e forse anche col secreto di ghiandole salivari. In queste specie, l'alimento delle larve consiste dunque esclusivamente di sostanze liquide o

My first observation on this singular method of feeding the larvae was made on a large nest of Pachycondyla found under a stone at the foot of Mt. Bonnell, near Austin, May 5. Before the ants could carry them away, I had scooped up a fine lot of larvae, together with the earth in which they were lying. Among the larvae were several pieces, one or two segments long, of a recently killed myriopod (Scutigera). Into these pieces the larvae, some of which were nearly full-grown, had inserted their heads and were devouring the softer tissues! This could be distinctly seen with the pocket lens through the glass of the vial to which the larvae had been transferred. In another nest of the same species, uncovered May 16, I observed the larvae in the nest lying on their backs, devouring the pieces of some insect which I could not identify.

The former of these observations made in the field led me to observe the feeding of the larvae in my artificial nest of Leptogenys. I had frequently wondered at the way in which these ants decapitated termite nymphs or cut off their abdomens and scattered these about among their larvae. It was all quite clear to me now; examination with the lens showed that the larvae had inserted their long necks through the cut surfaces into the soft parts of the termites and were feeding exactly like the larvae of Pachycondyla.

During the month of May I had frequent opportunity to see Odontomachus feeding its larvae in my artificial nests. These larvae are placed by the ants on their broad backs, and their heads and necks are folded over onto the concave ventral surface, which serves as a table or trough on which the food is placed by the workers. The following observations are transcribed from my notebook:

semiliquide; e tale é pure in massima l'alimento delle stesse formiche allo stato adulto, quando si cibano di sostanze zuccherine vegetali o degli escrementi liquidi degli afidi. Però, molte formiche vivono pure in parte di preda, e nulla prova che si contentino di sorbire i succhi della loro vittima, e non digeriscano pure, mediante la saliva, alcune parti solide." . . . "Ora sarebbe pure possibile che Formiche, le quale vivono principalmente di preda, diano in pasto alle loro larve pezzi più o meno triturati del corpo delle loro vittime come fanno le Vespe."

May 13. This evening several house-flies, placed in the Janet nest of *O. haematodes*, were at once shorn of their legs, then decapitated, and finally their thoraces and abdomens were cut into smaller pieces and distributed among the larvae. One was given a fly's head, which it kept twirling around in a comical manner, while it devoured the brain through the small cervical orifice. Another was given a piece of a thorax with one of the wings still attached, another a piece of an abdomen, still another, a leg with a mass of muscle at its coxal end, etc.

May 16. This evening a small homopterous insect was placed in the Odontomachus nest. One of the ants (A) snapped at it, disabled it, and then left it. A few moments later it was picked up by another ant (B) and carried into the chamber containing the larvae and pupae. Thereupon a third ant (C) took hold of it and began tugging at it with B till it was torn open, but not into pieces. B then placed it on the flat ventral surface of a medium-sized larva, which began feeding at once, moving the homopteron around with its jaws. After four minutes had elapsed, another ant (D) that had been standing near by, apparently much interested in the feeding, suddenly tore the morsel away and placed it on a small larva. This larva was permitted to feed ten minutes, closely watched during all this time by ant D and another (E) which had come up in the mean time. Then ant D tried to tear the morsel away from the small larva, but apparently unable to do so, it took up the larva with the morsel and dumped them both on the ventral surface of a large larva. This creature seized the homopteron and forced the small larva to release its hold and to drop to the ground. The large larva fed for fully twenty minutes, closely watched by ant D and two others (E and F). All of these ants tried at different times to wrench the morsel away from the larva, but failed. Suddenly a small ant (G) rushed up, tore it away, and ran off with it. By this time very little was left of the homopteron and I lost track of it.

May 23. A few crumbs of cake, moistened with water, were placed in the Odontomachus nest at 11.7 P.M. A worker soon carried one of the crumbs into the breeding chamber and gave it to a large larva at 11.20. This larva fed but a few moments, but the cake was not removed till 11.35, when it was carried into another chamber, then at once brought back and placed between three larvae, from one of which it had just been taken. The smallest of these three larvae nibbled at it for a short time, beginning at 11.40. But one minute later this larva was carried away by a worker, and the cake was taken by another worker and given to a small larva at 11.43. This larva, too, was soon carried away (at 11.48), and the cake was taken to a large larva, which would have none of it. It was not removed, however, till 11.50. Then it was given by another worker to a large larva, which did eat some of it. At 11.51 the piece of cake, but little diminished in size after all its perambulations, was taken to another large larva. The ant remained over the larva holding the cake in place till 11.58, when another worker came up and ran away with the larva. While the larvae were feeding, the ants themselves could be plainly seen to partake of the cake from time to time. During the whole period of the above observations, and for some minutes later, *i.e.*, for over an hour, one little larva was permitted to feed without interruption on what seemed to be a piece of a house-fly.

These observations lead us to several interesting reflections. First, it is certain that the feeding of the larva of the Ponerinae is of a far more primitive character than in any other ants in which this process has been studied. It is, in fact, even more primitive than the corresponding habit of the social wasps, which feed their larvae with masticated insect prey, for in the Ponerinae the prey is cut into a few pieces only, for the purpose of exposing the soft tissues and making them accessible to the mandibles of the larvae. Myriopods or large insects are disarticulated for this purpose, small insects are merely torn open. Leaving the question of systematic affinities out of consideration, the Ponerinae may be said to have habits of feeding the young intermediate between the habits of the solitary wasps, which provide their young with whole insects, and the social wasps, which masticate the food for their larvae. In this statement it may, perhaps, be more accurate to substitute the Bembecidae for the solitary wasps, since the Bembecidae, which feed their larvae from day to day with entire Diptera in a fresh condition, resemble the Ponerinae more closely than do the solitary wasps, which merely enclose their eggs with paralyzed larvae, spiders, grasshoppers, etc.[1] From the condition of the Ponerinae to that of the more specialized ants, which feed their larvae with nothing but the liquid food regurgitated from their own crops or from their salivary glands, the transition is very abrupt. But there are many ants whose habits have not been studied, and some of these may yet be found to bridge this chasm.

In the second place, the above-recorded observations seem to show that the Ponerine method of feeding the larvae is of a most capricious and irregular character. The quantity and quality of the food given to a particular larva, and the time it is permitted to feed, seem to be matters requiring no very strict regulation. The ants that feed the young rarely act in concert, but rather with a whimsical individualism that seems at times to border on the ridiculous.

This irregular method of feeding suggests other considerations of a wider bearing. It is generally admitted that the polymorphism of the female sex in ants, *i.e.*, the occurrence of fertile females and of sterile females of one or more casts, is in some manner correlated with the feeding of the larvae developed from fertilized eggs. In other words, the worker ants can control the production of individuals like themselves and of individuals like their queen. It is further maintained that these differences are effected by the quantity and quality of the food administered to the larvae at a certain period of their development; but here our knowledge ends. These data have been accumulated from the study of the specialized Myrmicine and Formicine ants of Europe and North America, and are supported by many valuable observations on the hive-bee. Now, while we can, perhaps, understand how these more specialized ants may manage to control the quantity and quality of liquid food regurgitated from their own crops and salivary glands, it is not so easy to understand how ants can exercise such control when they adopt a capricious method of feeding like that of the Ponerinae. Such a method can hardly produce clear-cut results; *i.e.*, either workers or fertile females. And a comparative study of the better known species of Ponerinae shows that in certain species at least there is no such sharp distinction between the sterile and fertile female as we find in the more specialized ants. Not only is the female sex in a state of morphological and physiological instability, — *i.e.*, di- or even tri-morphic, — but the male sex also is sometimes dimorphic — at least in the same genus, if not in the same species. For the purpose of illustrating this singular instability of the sexes I have compiled the following table from the literature to which I have access.[1] It includes twelve of the better known species

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[1] Fine descriptions of wasps (Polistes) and Bembecids feeding their young are to be found in the charming works of Fabre (Souvenirs Entomologiques, 1° ser., 2m edit., Paris, 1894, pp. 126–128 and pp. 226 *et seq.*) and of Dr. and Mrs. G. W. Peckham ("The Instincts and Habits of the Solitary Wasps," *Bull. Wisconsin Geol. N. H. Survey*, No. 2, 1899, 245 pages, 14 plates). Janet has described the corresponding habits of Vespa ("Études sur les fourmis, les guêpes, et les abeilles." 10. Note. Sur Vespa media, V. silvestris, et V. saxonica, *Mém. de la Soc. Acad. de l'Oise*, tome xvi, 1895, p. 39).

Species of Ponerinae.	Winged Male.	Ergatoid Male.	Winged Female.	Ergatoid Female.	Worker Major.	Worker Minor.
Odontomachus haematodes Linn	+		+	+	+	
Pachycondyla harpax Fabr. . .	+		+	+	+	
Cardiocondyla Emeryi Forel . .	+		+	+	+	
Cardiocondyla Wroughtonii Forel		+	+		+	
Cardiocondyla Stambulofii Forel		+	+		+	
Leptogenys elongata Buck. . .	+			+	+	
Ponera ergatandria Forel . . .	? +	+	+		+	
Ponera ochracea Mayr.	+		+		+	
Ponera Eduardi Forel	+		+	+	+	
Ponera coarctata Latr.	+		+	+	+	
Ponera punctatissima Rog. . .		+	+		+	
Stigmatomma pallipes Hald. . .	+		+		+	

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[1] Sharp, "Formicidae in Cambridge Natural History," *Insects*, vol. vi; Emery, "Sopra Alcune Formiche della Fauna Mediterranea," *R. Accad. delle Scienze dell' Istituto di Bologna*, 21 Apr., 1895; Emery, "Beiträge zur Kenntniss der nordamerikanischen Ameisenfauna," Schluss, *Zool. Jahrb.*, Abth. f. Syst., Bd. viii.

of Ponerinae. The presence of a particular sexual phase is indicated by a cross.

Although it is by no means certain that the irregular polymorphism of the two sexes of the Ponerinae, as indicated in this table, is due to an inability on the part of the ants to regulate with precision the quality and quantity of the food administered to the larvae, I nevertheless believe that there is some causal connection between these two peculiar phenomena. At any rate, we may assume this connection as a working hypothesis for future experimentation and observation. I believe that continued study of the relatively undifferentiated sexual conditions of the Ponerinae may lead us more rapidly to a solution of the interesting problems of nutritional polymorphism than a study of the more specialized ants.

When the larvae of the Ponerinae are mature they are, like the mature larvae of the Formicinae, buried in the soil till they have spun their cocoons. They are then unearthed and the small adherent particles of soil are carefully removed by the workers. I have watched the burying of the larvae in Leptogenys and the unearthing and cleansing of the cocoon in Odontomachus. The cocoons of the three species of Ponerinae are usually kept together, but the ants are scarcely as careful in this respect as the species of Formica and Camponotus which I have observed (*F. neorufibarbis* and *C. castaneus*). Nor do they keep their larvae assorted according to sizes, a peculiarity which accentuates the irregularity of their feeding habits.

Forel, as we have seen, believes that *Ponera coarctata* may escape from its cocoon without the assistance of the workers. Unfortunately I had to leave my work at Austin before the pupae of Odontomachus were ready to hatch, but I am convinced that Leptogenys, at any rate, opens the cocoon and draws out the pupa when ready to enter on its imaginal life. I have not seen this operation under normal circumstances, as the two workers which appeared as callows in my artificial nest left their cocoons when I was not present, but for some reason the workers in this nest were continually opening the cocoons near one end and pulling out the still white pupae. Ten or a dozen workers would gather about one of these extracted pupae and lick it for hours. Sometimes one ant would take possession of the limp thing and hold it astraddle for a long time. Ultimately these prematurely born ants were either devoured by the workers or fed to the ravenous larvae. Nevertheless the deft manner with which the cocoon was opened, the pupa extracted, and the empty cocoon at once placed on the kitchen-midden, or rubbish heap, indicated very clearly that this is also the method of procedure with pupae that have reached their full growth.

A word in conclusion concerning myrmecophiles, for the Ponerinae, like the other subfamilies of ants, are known to harbor arthropod guests in their nests.[1] No guests were taken with Odontomachus and Leptogenys, but some six different species were observed in various nests of *Pachycondyla harpax.* Only one of these had not previously been found in the nests of other species of ants near Austin. This was a small yellow ant, a Solenopsis, allied to the European *S. fugax*, and found inhabiting some very minute galleries in the earth between the huge burrows of the Ponerine. It is probably a "Diebsameise," given to myrmecoclepsy like its European congener. Two specimens of the very singular little ant, *Strumigenys louisianae* Rog., were also taken from the earth of this same nest. Their relations with the Pachycondyla were probably of a more accidental nature. The other forms taken are pleomyrmecophilous, *i.e.*, they occur in the nests of several other species of ants in the vicinity of Austin. These are, first, a yellowish white species of Lepismina, quite common in the nests of Pachycondyla, but even more abundant in the nests of *Camponotus castaneus* Latr., in the same localities. This Thysanuran was also taken in the nests of *Eciton coecum* Latr. Second, a white Collembolan, similar to, if not the same as, *Cyphodeira* (*Beckia*) *albinos* Nicol. of Europe. This insect is panmyrmecophilous, occurring in the nests of nearly all the ants of Travis County. Third, *Myrmecophila nebrascensis* Bruner, rare in the nests of Pachycondyla, but very common in the nests of *Formica fusca*, var. *neorufibarbis* Mayr. I have no doubt that this singular little cricket had strayed from the Formica to the Ponerine nests. Fourth, a small Trichopterygid beetle was sometimes found in the nests of Pachycondyla. As this same species was very common in the nests of *Camponotus castaneus*, in the same localities, I believe that it, too, may have strayed from the nests of its typical host.

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[1] The Ponerine guests enumerated by Wasmann in his Kritisches Verzeichniss der myrmekophilen und termitophilen Arthropoden, Berlin, 1894, are the following: *Typhloponemys hypogaea* Rey (staphylinid beetle), with *Typhlopone oraniensis* Luc., Palestine; *Apocellus* (?) *sphaericollis* Say (staphylinid), with *Ponera coarctata* Latr., North America; *Mesotrochus paradoxus* Wasm. (staphylinid), with *Typhlomyrmex Rogenhoferi* Mayr, Santa Catharina; *Euplectus Sikorae* Wasm. (pselaphid beetle), with *Ponera Johannae* Forel, Madagascar; *Trichonyx sulcicollis* Rchbch. (pselaphid), with *Ponera coarctata* Latr., Europe; *Amauronyx Märkeli* Aubé (pselaphid), with *Ponera coarctata* Latr., Switzerland; *Araniops amblyoponica* Brend. (pselaphid), with *Stigmatomma pallipes* Hald., Pennsylvania, North Carolina; *Tmesiphorus formicinus* McL. (pselaphid), with *Ectatomma sociale* McL., Australia; *Leptotrichus inquilinus* Koelbel (isopod crustacean), with *Ponera senarensis* Mayr, East Africa. More recently Wasmann has described the following ponerinaphiles: *Fauvelia permira* Wasm. (staphylinid), with *Pachycondyla Fauveli* Emery, Bolivia ("Die Ameisen- und Termitengäste von Brasilien," 1. Theil, *Verhandl. d. k. k. zoöl. bot. Gesell.*, Wien, Jahrg. 1895, pp. 40, 41); *Lomechon Alfaroi* Wasm. (silphid), with *Pachycondyla aenescens* Mayr, Costa Rica ("Ein neues myrmekophiles Silphidengenus aus Costa Rica," *Deutsch. Ent. Zeitschr.*, Heft. ii, 1897); *Myrmedonia lobopeltina* Wasm. (staphylinid) and *Demera Fauveli* Wasm. (staphylinid), with *Leptogenys* (*Lobopelta*) *nitida* Sm., Natal ("Zwei neue Lobopelta-Gäste aus Südafrika," *Deutsch. Ent. Zeitschr.*, Heft. ii, 1899).

UNIVERSITY OF TEXAS MEDICAL SCHOOL,
GALVESTON, June 10, 1900.

THE EYES OF THE BLIND VERTEBRATES OF NORTH AMERICA. III.

THE STRUCTURE AND ONTOGENIC DEGENERATION OF THE EYES OF THE MISSOURI CAVE SALAMANDER, AN ACCOUNT BASED ON MATERIAL COLLECTED WITH A GRANT FROM THE ELIZABETH THOMPSON SCIENCE FUND.[1]

CARL H. EIGENMANN AND WINFIELD AUGUSTUS DENNY.

A SINGLE specimen of a salamander was discovered in Rock House Cave, Barrie County, Missouri, by Mr. F. A. Sampson in July, 1891. The specimen was described by Stejneger in the *Proceedings of the U. S. National Museum*, Vol. XV, p. 115, as Typhlotriton spelæus. His diagnosis reads as follows: "Vertebrae opistocœlous; parasphenoid teeth; vomerine teeth; eyes concealed under the continuous skin of the head; tongue attached in front and along the median line, free laterally and posteriorly; maxillar and mandibular teeth small and numerous; vomerine teeth in two strongly curved series; parasphenoid patches separate; nostrils very small; toes five; sixteen costal grooves, or eighteen if counting the axillary and groin grooves; tail slightly compressed, not finned; toes nearly half webbed; vomerine teeth in two *V*-shaped series with the curvatures directed forward; gular fold strong, very concave anteriorly; color uniformly pale."

Stejneger fully appreciated the value and nature of his discovery. He says: "Although many of our salamanders are known to inhabit caves, this seems to be the only one, so far discovered, which, like some of the other animals exclusively living in caves, has become blind or nearly so." This was written by him before he discovered the Typhlomolge in the underground streams of Texas.

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[1] *Contribution from the Zoölogical Laboratory of the Indiana University, No. 31.*

A preliminary note by the present authors (*Proc. Ind. Acad. Sci., 1898*, p. 252, 1899) completes the list of papers dealing with this species.

In the spring of 1897 Dr. Eigenmann visited Rock House Cave and secured a number of larvae, which Dr. Stejneger pronounced the larvae of Typhlotriton. Later Mr. E. A. Schultze informed him that he had seen this salamander in the underground passage to Blondi's Throne Room in Marble Cave, Stone County, Missouri. In September of 1898 he visited this cave and secured four adults and three larvae of Typhlotriton. A large number of the larvae were obtained from Rock House Cave a few days later. Those from the latter cave were found under loose stones and gravel in the rivulet at the mouth of the cave. They had been exposed to the light. It is scarcely supposable that those from Marble Cave had ever been affected by the light. In the caves both larvae and adults are found under the stones, the old ones in and out of the water. Occasionally one is seen lying on the bottom of a pool.

In the aquarium the larvae creep into or under anything available; a glass tube serves as a "hiding" place. The rubber tube admitting water to the aquarium is sometimes occupied by several during a temporary cessation of the flow of water. A wire screen sloping from the bottom of the aquarium formed the most popular collecting place for the larvae. They collected beneath this, although it was no protection from the light. From these observations it seems probable that stereotropism rather than negative heliotropism accounts for the presence of this species in the caves, and that this reaction has been retained after the long stay of the species in caves necessary to account for the changes in its eyes.

The eyes of the larvae when examined from the surface appear perfectly normal, but they are little used in distinguishing objects. When hungry they will strike at a stick held in the hand as they would at food. A stick lying at the bottom of the aquarium undisturbed is not molested. They strike at a worm when touched by it, or when it approaches close enough for its motion to be perceived.

In the larvae up to 90 mm. long the skin passes over the eye without forming a free orbital rim and the eye does not protrude beyond the general contour of the head. In the adult from 97 mm. on, the eye forms a bead-like projection. There are in the adult distinct lids. These are closed over the eye, covering it entirely, the slit being much too small for the eye. The lower lid is free from pigment, but the upper lid, which closes over the lower, is as thickly pigmented as any other part of the body.

Stejneger says of the eyes that they are "small, only slightly raised, and covered by the continuous skin of the head, with only a shallow groove to indicate the opening between the lids, the underlying eyes visible as two ill-defined dusky spots."

In sections the lids are seen to overlap one another some distance, forming an obscure, free orbital rim. Fig. 1, *a*, is a median section of the lids and corneal epithelium of an eye .954 mm. in diameter, taken from an adult specimen 106 mm. in length. In this section the upper lid overlaps the lower lid .216 mm., or more than one-fifth the diameter of the eye. Passing from the median section toward the corners of the eye, the lower lid unites with the underlying tissue first. When observed from the top the upper lid covers the entire eye. The orbital slit is .17 mm. in length. The conjunctival pocket extends some distance forward and backward beyond the slit. The eye increases in size but little from the larval to the adult stage, and its growth is not proportional to the growth in length of the animal. (See comparative measurements of the eyes at the close of the paper.)

The following is a series of measurements on the larvae of Typhlotriton.

	Rock House Cave.	Rock House Cave.	Marble Cave.
Specimen . . .	54 mm. long.	78 mm. long.	88 mm. long.
Size of pupil . .	.432 mm.	.640 mm.	———
Length of eye . .	1.30 mm.	1.50 mm.	1.60 mm.
From optic nerve to front of lens .	.80 mm.	1.20 mm.	———
Vertical diameter	———	1.248 mm.	1.28 mm.

Sections of the adult and larva from Marble Cave were made in the usual manner. The six normal eye muscles were present in Typhlotriton. The m. recti form a sheath about the optic nerve in its distal part and spread out from it near the eye. In the adult the sclera is a layer of uniform thickness except in the region of the entrance of the optic nerve. It is not usually separated from the adjoining parts of the eye, but in places is retracted a short distance from the choroid coat by the action of reagents. It is for the most part fibrous, with few compressed nuclei, and varies from 18 to 40μ in thickness. In the larva a narrow cartilaginous band surrounds all but the ventral wall of the eye. In a specimen 35 mm. long the width of the band is about 30μ, its thickness 16μ. In three adult specimens the sclera of only one had any traces of cartilage. In the right eye of the adult specimen 103 mm. long a cartilage about 36μ thick, 60μ wide, and not more than 40μ long is found on the upper face of the eye. The absence of this cartilage in the adult has probably no connection with the degeneration of the eye. Its presence is probably a larval characteristic which disappears as the gills disappear during the metamorphosis.

The average thickness of the cornea is 40μ. In the adult it is covered by a layer of stratified epithelium, 25μ in thickness, consisting of three rows of cells. The cells of the inner row are columnar in shape, those of the middle row rounded, and those of the outer row are very much flattened and elongated (Fig. 1, *a*).

In the adult the choroid coat is usually separated from the pigment layer, but adheres closely to the sclera. In general it is thicker at the back part of the eye, and quite decidedly so at the entrance of the optic nerve. The lens is normal. Its size is given in the table at the end of the paper.

The layers of the retina are well developed in the larva. The retina of the larva differs from that of an Amblystoma larva in the greater thickness of its ganglionic layer. This layer is, in the young larva of Typhlotriton, composed of five or six layers of cells. This thickness may in part be an artifact, since the retinae examined are shrunken away from the pigment epithelium, and the ganglionic layer is in contact with the lens. In the larva 90 mm. long this layer has been reduced to not more than three series of cells. Aside from the differences noted above, the eye of the larvae of Typhlotriton is apparently normal in all of its histological details. This relative thickness in the different sizes of the larvae may be gathered from Figs. 2–5 and from the comparative table at the end of the paper.

Figs. 2–5 are drawn with the same magnification and show the relative thickness of the different layers in the retinae of the larvae of different sizes and of the adult. The adult retina is reduced in thickness by the absence of the rods and cones and the (partial ?) atrophy of the outer reticular layer and by the thinning of the ganglionic layer. The ganglionic layer in the adult contains from two to five rows of cells. In this respect, the adult approaches the condition found in Amblystoma more than the young does. The inner reticular layer is comparatively thick, that of the young being thicker than that of the adult.

In the adult the inner nuclear layer is continuous with the outer nuclear layer. (See Fig. 5.)

The inner nuclear layer consists of about seven series of cells in the smallest larva and of four to seven in the largest. The cells in the preparations available cannot be separated into bipolar and spongioblastic layers, nor are horizontal cell layers distinguishable. The outer reticular layer is well differentiated but quite thin in the larvae, and is irregular in outline, adapting itself to the overlying nuclei which encroach on its outlines. In the adult this layer is indistinguishable by the same methods that make it conspicuous in the larva. In places there appeared an open space where the outer reticular layer should be (Fig. 9), but none of its structure remains. It is fair to suppose that the fibers forming this layer are resorbed during the metamorphosis. This layer seems to be the very first obliterated by the processes of degeneration both ontogenic and phylogenic in this as in other vertebrates with a degenerating eye.

The greatest change during and shortly after metamorphosis takes place in the layer of the rods and cones. In the larva 35 mm. long, from the mouth of Rock House Cave, the rods reach an extreme length of 50 μ. The relative sizes and number of these as compared with the much smaller cones may be gathered from Fig. 2, *a*.

In the larva 90 mm. long the outer segments of the rods are much shorter and stain less conspicuously than in the younger. The nuclei of the outer nuclear layer are distinctly in two layers, whereas in the younger they are in three less regular layers. The cones are correspondingly fainter than in the young. It is surprising that whereas in the larva 90 mm. long we find the rods and cones well developed they have greatly degenerated or practically disappeared in the adult only a few mm. longer. In an adult specimen 97 mm. long the rods have retained their normal shape and position, but I have not been able to detect any differentiation into inner and outer segments. In longer ones most of the nuclei of the outer series have become rounded at both ends. But one cone was found in eyes of the adult over 100 mm. long. It is shown in Fig. 6. In an adult specimen 103 mm. long filmy rods are still evident. They appear as conical spaces above the nuclei free from pigment rather than as possessing any demonstrable structure. Just at the margin of the place where the pigment has been torn from the retina one of these is drawn out to a great length. The pigment in this individual extends in places down between the cells of the cones. This latter condition appears in a very exaggerated form in the eye of Typhlomolge. In tangential section this condition and the filmy rods give rise to the appearance represented in Fig. 5, *a*.

Distinct signs of ontogenic degeneration are also seen in other parts of the retina. For instance, many nuclei of the inner series of the outer nuclear layer are shriveled. In some eyes the ganglionic nuclei have for the greater part lost their granular structure and show a homogeneous pasty condition, only a few cells with granular nuclei being present (Fig. 5). The same is true in large part of the inner nuclei of the inner nuclear layer. This condition of the ganglionic nuclei is not entirely confined to the adult but is also found in the larva.

Some of the modifications in the shapes of the outer nuclei in the adult are shown in the figures. In Fig. 7 the upper portion of the nucleus is very much elongated. This form is of frequent occurrence. In Fig. 8 is shown the common form where the nuclei are simple elliptical bodies, which give no evidence whatever of any processes uniting them with the other elements of the retina. The Müllerian fibers are profusely present and of very large size in both larva and adult. In both adult and young the optic nerve enters as a single strand and passes entirely through the layers. A heavy mass of pigment is found following the optic nerve to within a short distance of the brain.

Average Measurements of the Eyes of Typhlotriton.

Length of Specimen.	35 mm.	48 mm.	62 mm.	90 mm.	97 mm.	103 mm.	106 mm.
Vertical diameter of eye	810	800	—	960	—	800	1170
From front of lens to back of eye .	600	672	—	720	720	720	1134
Outer nuclear layer with the rods .	76	42	112	36	28	28	—
Outer reticular layer	1	2	—	—	—	—	—
Inner nuclear layer	76	72	80	50	48	72	72
Inner reticular layer	16	20	16	24	8	8	13
Ganglionic layer	68	56	64	32	24	26	26
Pigment layer	4	16	—	—	8	20	22
Optic nerve	20	25	—	—	—	23	29
Lens	342	300	—	500	432	430	504

Summary.

Typhlotriton is an incipient blind salamander living in the caves of southwestern Missouri. It detects its food by the sense of touch without the use of its eyes. It is stereotropic. Its eyes show the early stages in the steps of degeneration from those of salamanders living in the open to those of the degenerate Typhlomolge from the caves of Texas. The lids are in process of obliteration, the upper overlapping the lower so that the eye is always covered in the adult. The sclera possesses a cartilaginous band in the larval stages but not in the adult. The disappearance of the cartilage is probably an incident of the metamorphosis, not of the degeneration the eye is undergoing. The lens is normal. The retina is normal in the larva with a proportionally thicker ganglionic layer than in the related epigæan forms. Marked ontogenic degenerations take place during and shortly after the metamorphosis. *a.* The outer reticular layer disappears. *b.* The rods and cones lose their complexity of structure, such as differentiation into inner and outer segments, and finally are lost altogether.

EXPLANATION OF FIGURES.

All drawings were made with the aid of the Abbe camera from sectioned balsam preparations. The comparative measurements (p. 39) furnish the key to the magnification:

ps.	palpabra superior.	*pi.*	palpabra inferior.
1.	pigment epithelium.	2.	rods and cones.
3.	outer nuclear layer.	4.	outer reticular layer.
5.	horizontal cell layer.	6.	inner nuclear layer.
7.	spongioblastic layer.	8.	inner reticular layer.
9.	ganglionic layer.	10.	optic fibers.

FIG. 1. Diagrammatic representation of the eye drawn to scale.

FIG. 1, *a.* Vertical section through the cornea and lids of an adult.

FIG. 2. Section of the retina, exclusive of pigment cells, of a larva 35 mm. long.

FIG. 2, *a.* Tangential section through the rods and cones about on a level with the innermost extent of the pigment which is seen on the right, showing the relative sizes and abundance of the rods and cones.

FIG. 3. Section of the retina of a larva 48 mm. long.

FIG. 4. Section of the retina of a larva 90 mm. long.

FIG. 4, *a.* Tangential section showing the rods and cones at about the inner limit of the pigment which is seen on the left.

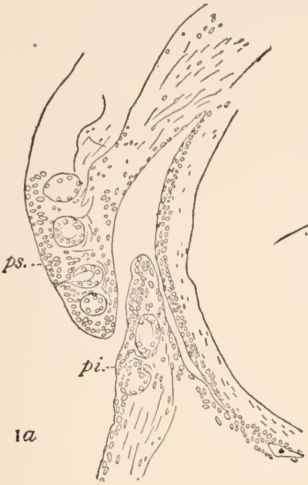
FIG. 5. Section of the retina of an adult 106 mm. long.

FIG. 5, *a.* Tangential section at about the inner limit of the pigment.

FIG. 6. The only cone found in the eyes of adults.

FIG. 7, 8. Difference in the shape of the outermost series of cells in the outer nuclear layer.

FIG. 9. Section of the retina of an adult 97 mm. long.



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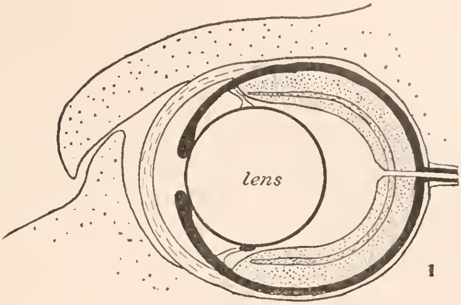

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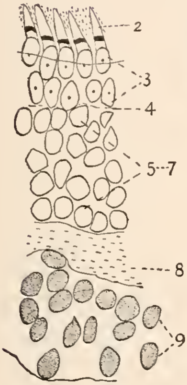

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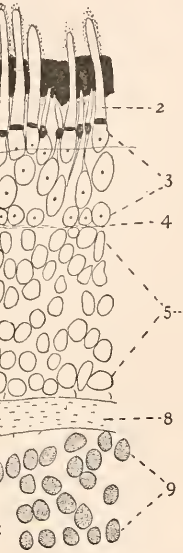

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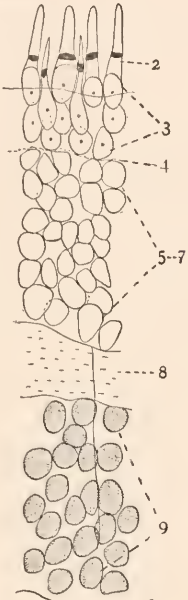

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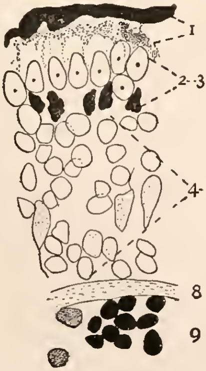
5a



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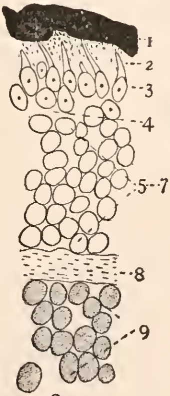

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Volume II.] *November, 1900.* [*No. 2.*

BIOLOGICAL BULLETIN.

THE HABITS OF PONERA AND STIGMATOMMA.[1]

WILLIAM MORTON WHEELER.

In a recent number of the *Biological Bulletin*[2] I described the habits of three Texan ants belonging to the subfamily Ponerinae. During the past summer an excellent opportunity presented itself to extend these observations to two other forms widely distributed in the Eastern and Northern States, *viz.*, *Ponera coarctata* Latr. and *Stigmatomma pallipes* Hald. These are of no little interest to the student of ant life, the former as a member of the typical genus, the latter as the only known North American representative of the most primitive tribe (Amblyoponii) of the subfamily. European myrmecologists have long wished to gain some knowledge of the habits of *P. coarctata*, but its rare and local occurrence on their continent has rendered this impossible up to the present time. The European type of the genus *Stigmatomma*, *S. denticulatum* Roger, is also rarely seen, and for the same reason its habits are all but unknown.

As both the ants to be considered in this paper are subterranean and very timid, it is impossible to learn much about them in their natural environment. It is therefore necessary to keep them in artificial nests. This is, fortunately, an easy matter, since the ants are very hardy. As the colonies are small, it suffices to use for this purpose the Petri dishes employed by bacteriologists for growing cultures of micro-organisms. The ants are hastily scooped up, together with their larvae, pupae, and much of the earth in which they have excavated their nest, and the whole is transferred to a Petri dish. One or two glass slides are then placed on the earth, which is spread out till it forms a layer not more than about 5 mm. in thickness. The Petri dish is kept covered to retain the moisture in the soil. In the course of a day or two the ants excavate rough-walled chambers under the slide and galleries in the adjacent soil, of the same size and shape as those which they are in the habit of forming in their natural nests. They also gather their eggs, larvae, and pupae into these chambers, where they may be easily seen. When the slides become smeared or covered with earth they can at any time be hastily replaced by clean ones without greatly disturbing the ants.

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[1] *Contributions from the Zoölogical Laboratory of the University of Texas*, No. 10.

[2] "A Study of some Texan Ponerinae," *Biol. Bull.* Vol. ii, No. 1, pp. 1–31. October, 1900.

The Ponerinae may appear to lead very monotonous lives to any one who has kept under observation the different species of *Myrmica*, *Pogonomyrmex*, *Lasius*, *Camponotus*, and *Formica*. But this very monotony is full of interest to the observer who sees in the rudimental activities of these ants a certain picture, however imperfect, of the simple stages through which the higher ants have passed in attaining to their present remarkably differentiated social organizations. It can hardly be doubted that there is a phylogeny of instincts, as there is a phylogeny of structures, and there is certainly no single animal group which more clearly illustrates the truth of this statement than the Formicidae.

PONERA COARCTATA LATREILLE.

Our American *P. coarctata* is considered by Emery[1] to differ sufficiently from the European form to be ranked as a subspecies, which he calls *pennsylvanica* Buckley. In the worker the single node forming the pedicel of the abdomen is somewhat thicker and much broader behind and less narrowed anteriorly than in the European forms. The punctation of the head is finer, that of the thorax and node much denser and more distinct. Emery also mentions some differences in the neuration of the wings of the male: "in den Flügeln verbindet sich aber die *Costa recurrens* etwas weiter von der Gabelung mit dem hintern Ast der *Costa cubitalis*, ungefähr wie bei der europäischen *P. punctatissima*."

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[1] "Beiträge zur Kenntniss der nordamerikanischen Ameisenfauna" (Schluss), *Zool. Jahrb.* Abth. f. System. Bd. viii, pp. 257-360, Taf. VIII. 1894.

Figs. 1, 2, and 3, from camera drawings, represent the outlines of the male, female, and worker of the American *coarctata*. The eyes of the worker are minute and vestigial, those of the female considerably and those of the male very much larger. The worker has no ocelli; those of the female are small, while in the male they are very prominent. The node in the male and female is more slender than that of the worker, and of a somewhat different shape. The antennae of the male are of nearly uniform thickness throughout and 13-jointed, whereas the geniculate antennae of the female and worker are 12-jointed, with a long basal joint, or scape, and a club-shaped funicular portion, with much shortened middle joints. The worker and female are provided with a long sting; while the pygidium of the male ends in an acute point.

The coloration of the female and worker is highly variable. Typical specimens have the head, thorax, node, and base of the abdomen black, the mandibles, clypeus, frontal carinae, antennae, legs, posterior third of the first abdominal segment, and the tip of the abdomen from the base of the fourth segment, red or yellow. Very often the ventral portions of the trunk are more or less suffused with red or yellow, especially when the specimens are immersed in alcohol. Some specimens, probably more or less immature, are red or yellow throughout. The body is covered in all cases with short pale pubescence, which on the head forms two lines, one on either side running parallel with the straight lateral edges. These lines are apparent only in dry specimens seen in a certain light. The male is black, with the palpi, trochanters, knees, tips of tibiae, and the tarsi light yellow. The genitalia and the incisures of the segments of the slender abdomen are also more or less yellow or piceous, as are also the stigma and veins of the colorless wings, both in this sex and in the female.

P. coarctata is a small ant, the male and female measuring scarcely more than 4 mm. in length, while the workers vary from 3 to 3.75 mm.

According to Emery this ant occasionally presents ergatoid females. He mentions[1] two of these wingless individuals from Sicily, with eyes somewhat larger than those of the worker, with ocelli and with the node somewhat higher and more slender above. I have been unable to find any such specimens among my American material, although I carefully scrutinized no less than two hundred wingless individuals from widely separated localities and from at least twenty different nests.

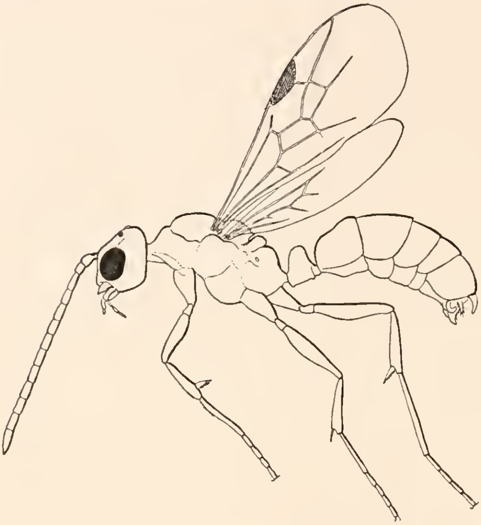

FIG. 1. — *Ponera coarctata* Latr., subsp. *pennsylvanica* Buckl. Male.

P. coarctata is the most widely distributed of the European Ponerinae and occurs even in northern Africa (Algiers), according to Emery.[2] In this country, too, its subspecies, *pennsylvanica*, is one of the most widely distributed forms in the subfamily. Emery[3] has examined specimens from Pennsylvania, New Jersey, Virginia, Maryland, Mississippi, Florida, and Ohio. Forel has observed it in North Carolina,[4] and I can add to this list four other states, *viz.*, Wisconsin, Illinois, Massachusetts, and Connecticut. It may, I think, be safely said to inhabit all the states east of the Mississippi, as well as Canada.

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[1] "Sopra Alcune Formiche della Fauna Mediterranea," *Mem. letta alla R. Accad. delle Scienze dell' Istituto di Bologna.* Pp. 1–19, Tav. I. 21 Aprile, 1895.

[2] *loc. cit.*, p. 6.

[3] "Beiträge zur Kenntniss," etc., *loc. cit.*, p. 268.

[4] *Annales de la Soc. Entomol. de Belgique.* Tome xliii, pp. 438–447. 1899.

It is undoubtedly far more common in this country than in Europe. In July I found numerous nests at Rockford, Ill., both under the bark of old logs and under stones along the streets of the town. It is not uncommon in similar locations at Woods Holl, Mass., and very abundant under stones on the slopes of Mt. Pisgeh (altitude 1450 feet), at Colebrook, Litchfield County, Connecticut.[1]

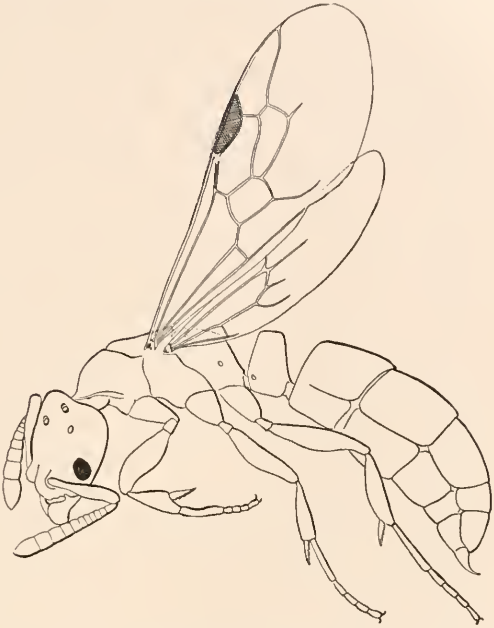

Fig. 2. — *Ponera coarctata* Latr., subsp. *pennsylvanica* Buckl. Virgin female.

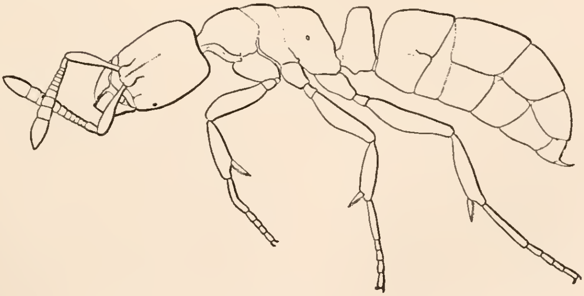

Fig. 3. — *Ponera coarctata* Buckl., subsp. *pennsylvanica* Buckl. Worker.

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[1] This last locality, together with the slope of a small neighboring hill, is a rich collecting ground for ants. I give here the complete list of the forms taken there during August, as it probably embraces nearly all the species of Formicidae that occur in New England: *Brachymyrmex Heeri* Forel, subsp. *depilis* Emery; *Lasius niger* L.; *L. flavus* L.; *L. umbratus* Nyl., subsp. *mixtus*, var. *aphidicola* Walsh; *L. latipes* Walsh; *Formica sanguinea* Latr., subsp. *rubicunda* Em.; *F. exsectoides* Forel, var. *opaciventris* Em.; *F. pallide-fulva* Latr., subsp. *Schaufussi* Mayr; *F. pallide-fulva*, subsp. *nitidiventris* Em.; *F. fusca* L., var. *subsericea* Say; *F. fusca*, var. *subaenescens* Em.; *F. fusca*, subsp. *subpolita* Mayr, var. *neogagates* Em.; *Camponotus herculeanus*, subsp. *ligniperdus* Latr., var. *novaeboracensis* Fitch (= *pictus* Forel); *C. herculeanus*, subsp. *pennsylvanicus* de Geer; *Stigmatomma pallipes* Hald.; *Ponera coarctata* Latr.,

P. coarctata is not found in deep woods or in damp places, but prefers rather dry localities more or less open to the sunlight. The margins of woods and along stone walls are favorite haunts, under stones rather deeply imbedded in a rich soil, especially leaf mold. Here it excavates a small, irregular chamber, from which a few straggling burrows run off into the neighboring soil. In some cases the chamber and burrows are found under the lower surface of the stone, but I have gained the impression, from the examination of many nests, that the ants often prefer the vegetable mold nearer the surface, where it overlaps the sides of the stones. Chambers and galleries of the same irregular pattern are excavated in the rotten wood when the ants nest under the bark of old logs. The larvae and pupae are reared in the chambers. In late June and early July the nests contain eggs and larvae but no pupae; during the latter half of July and the month of August only cocoons are found, usually crowding the chamber so that the ants have little space in which to move over and among them. The imagines begin to hatch during the last two weeks of August and the first week of September. Even by the latter date I have seen no eggs nor larvae to represent a second brood.

The number of individuals composing a colony varies in different nests and with the advance of the summer. As the ants are very timid and at once seek refuge in their galleries as soon as the stone that covers the nest is moved, it is not easy to determine their precise numbers. None of the nests opened at Rockford, Ill., July 1, contained more than eight or ten ants, including a single female. As soon as the cocoons begin to hatch, the colony increases rapidly. One rather typical nest, opened at Colebrook, Conn., August 24, contained six males, one female (with wing stumps and evidently the mother of the colony), one callow virgin female, twelve

subsp. *pennsylvanica* Buckl.; *Myrmicina Latreillei* Curt., subsp. *americana* Em.; *Formicoxenus nitidulus* Nyl.; *Solenopsis molesta* Say; *Crematogaster lineolata* Say, var.; *Stenamma* (*Aphaenogaster*) *fulvum* Rog., subsp. *aquia* Buckl., var. *piceum* Em.; *Myrmica rubra* L., subsp. *scabrinodis* Nyl., var. *Schencki* Em.; *Tapinoma sessile* Say.

workers, and forty-four cocoons. A few nests examined somewhat later in the month contained a greater number of individuals, so that fifty to sixty is perhaps not too great an estimate for a large colony by the first week in September.

The winged males undoubtedly leave the nests like the males of other ants, as I have taken them in the sweep-net in the grass while collecting small Diptera. I have also seen the males copulating with the newly hatched females in the same nest. The small size of the nests in the early summer would seem to indicate that the large number of workers in the late summer and early autumn must split up into several detachments, each with a young queen, and migrate to different localities. My reasons for making this statement, apart from the above-mentioned mating of the young queens within the parental nest, are largely of a negative character, but they may be given for what they are worth. First, I have observed no tendency in the young queens, while they possess wings, to leave the nests like the males; second, the wings are often lost very soon after hatching, sometimes before the queen has acquired her deep adult coloration; and, third, I have never found a solitary queen in the act of founding a nest, either of this or of any other of the five species of Ponerinae I have studied, although I have frequently seen the young fertilized queens of *Camponotus*, *Formica*, *Lasius*, *Tapinoma*, *Crematogaster*, *Stenamma*, *Myrmica*, and *Pogonomyrmex* starting their colonies. The fact that the colonies seem to be annual instead of perennial growths, as among other ants like those above mentioned, is of considerable interest. It points to very primitive conditions in the Ponerinae, especially as the same is also true of tropical forms like *Pachycondyla* and *Leptogenys*, which can hardly be destroyed by severe winters. Thus what was at one time erroneously supposed to be true of the more specialized ants, *viz.*, the founding of a colony by a young female leaving the parental nest like the young queen of the hive bee, accompanied by a number of workers, may prove to be the normal method of nest formation with the Ponerinae. If this supposition is correct, there must be considerable inbreeding in the colonies of these ants, as the females would be regularly fertilized by males from the same nest. There may be some connection between this condition and the limited productivity of these ants, and the strong tendency to parthenogenesis seen in some of the species (*e.g.*, in the ergatoid females of *Pachycondyla harpax*).

The behavior of *P. coarctata* towards individuals of the same species from different nests is very similar to that observed in *Pachycondyla.* If two nests be thrown together into the same dish, there may be no immediate signs of hostility ; but after a few hours have elapsed, the ants are found struggling together in pairs. They interlock mandibles or tug at each other's legs and antennae, or even wrestle fiercely, intertwining their long bodies and trying to use their slender stings. These contests may be renewed from time to time for many days, whenever two individuals from different nests happen to meet, but deaths are rare, and ultimately the colonies fraternize completely. Long before the ants have settled their various difficulties, however, the cocoons and larvae of both nests are brought together as common property. A dozen different nests can be compounded quite as easily as two, and a few ants from one nest can be induced to adopt a large number of cocoons and larvae taken from half a dozen different nests.

The eyes of the workers of *P. coarctata* are so very small that they can hardly be of much service as visual organs. The actions of the ants indicate that they are guided very largely by their extremely sensitive antennae. They are, undoubtedly, able to detect the difference between light and darkness, but the fact that they do not seem to mind exposure to the light, provided they are covered with a glass plate, leads me to infer that they are rather positively stereotropic than negatively heliotropic. Of course their preference for an atmosphere charged with a certain amount of moisture — their positive hygrotropism — leads them to seek refuge in dark places, under stones or the bark of old logs.

I have not been able to ascertain the food of these ants in a state of nature. They probably kill and imbibe the juices of very small subterranean insects. In captivity they are omnivorous, feeding readily on raw or boiled meat, yolk of eggs, corn bread, or even on "Boston brown bread." They do not appear to share the fondness of ants in general for sugar dissolved in water. When kept for a time without food they eat their dead companions or their own eggs, larvae, and pupae.

The workers of *Ponera* are never seen feeding one another with regurgitated food, like the different species of *Formica*, *Lasius*, and *Myrmica*. Even the queen is obliged to feed herself. The workers bestow on her no special attentions, nor does she enjoy any of the privileges of the queens of the above-mentioned specialized genera, after they have once established their colonies. Like any one of the workers, she takes part in digging the galleries, wanders out in search of food, assists in transporting and cleansing the eggs, larvae, and cocoons, and in feeding the larvae. Although not expressly stated in my former paper, this is also true of the ergatoid females of *Leptogenys* and *Pachycondyla*. This would seem to indicate a decidedly primitive condition, since the activities of the females of the Ponerinae never pass beyond the stage exhibited by the females of the more specialized ants only while they are raising their first batch of workers.[1]

In the scrupulous care of their nests, colonies of *P. coarctata* closely resemble the more specialized ants. They bury their food or any liquid or strong-smelling substance in their environment, and all refuse — dead ants, dead pupae, empty cocoons, etc. — is deposited in one corner of the nest.

The eggs of *P. coarctata* are oblong, like those of the other Ponerinae I have described (*Pachycondyla*, *Leptogenys*), and of very large size — fully .6 mm. long, or nearly as large as the thorax of the insect that lays them. The number produced at one time is, however, relatively small. Only three were deposited by one female in my nests July 20. As the larvae found in nests in early July are of very different sizes, we must assume that the queen lays a few eggs at a time, probably at intervals of a few days or a week. It is quite possible that some of the workers, acting as ergatoid females, may contribute unfertilized eggs which give rise to the males that are found in nearly every colony late in August.

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[1] In this connection it is interesting to note that, as Janet has shown ("Nids artificiels en plâtre. Fondation d'une colonie par une femelle isolée," *Bull. Soc. Zoöl.*, tome xviii, p. 168, France, 1893), the female of the more specialized ants, when separated from all her workers, may return to and repeat all the activities which she displayed while founding her first colony.

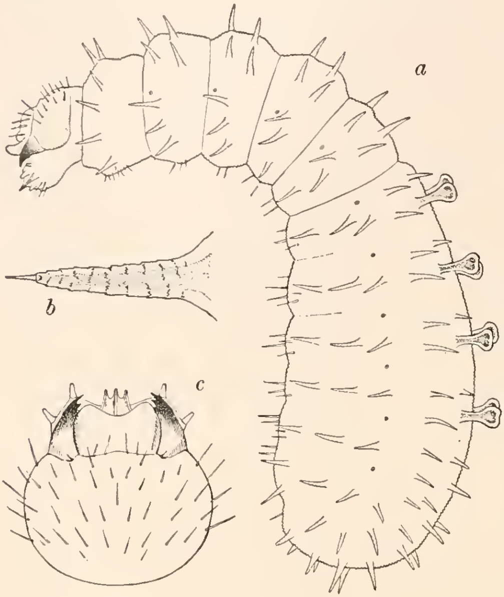

FIG. 4.—*a*, larva of *Ponera coarctata* Latr., subsp. *pennsylvanica* Buckl. Nearly ready to pupate. *b*, bristle-capped tubercle of same; *c*, head of same (dorsal aspect).

The larva (Fig. 4, *a*) is clearly of the Ponerine type, though differing in a few important particulars from any of the larvae of the five genera (*Leptogenys*, *Pachycondyla*, *Ponera*, *Odontomachus*, and *Diacamma*) described by Emery[1] and myself.[2] It is rather robust, with a large head succeeded by five distinct segments. The remaining segments, forming the swollen abdomen, are not distinctly marked off from one another. The body is furnished with outgrowths of three different types. The first of these is represented by a number of pointed bristles confined to the ventral surface of each segment. The second type is represented by several longitudinal rows of pointed tubercles, each of which, under a high magnification (Fig. 4, *b*) is seen to consist of a short distal spine and a long, tapering proximal base, directly continuous with the integument of the larva, and covered with transverse rows of serrated points. The distal spine is movably articulated with the proximal portion, and is so easily detached that it may be

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[1] "Intorno alle Larve di Alcune Formiche," *Mem. letta alla R. Accad. delle Scienze dell' Istituto di Bologna.* Pp. 1–10, 2 Tav. 7 Maggio, 1899.

[2] *Loc. cit.*, pp. 15–22.

overlooked. The third type of projection is found only on the dorsal surface of the third to sixth abdominal segment as four pairs of club-shaped structures which are glutinous to the touch. That these are peculiar modifications of the tapering tubercles seems to be indicated by the fact that they replace on either side in each of the four above-mentioned segments the more posterior of the two pointed projections seen in the thoracic, first and second, and seventh and eighth abdominal segments. The larva is usually kept on its back, so that the four pairs of glutinous tubercles act as suckers and fix it to the sides of the earthen chamber or to the glass of the artificial nest. The ants have to exert a slight effort in pulling the larva away from its attachment. The head of the larva in dorsal view is shown in Fig. 4, *c*. It is broad, evenly rounded behind, and beset with short stiff bristles. The labrum is bilobed and does not extend beyond the tips of the powerful tridentate mandibles. The fleshy maxillae and labrum project somewhat beyond the mandibles, the former being provided with robust tactile cones, the latter with a prominent median tubercle on which opens the duct of the spinning gland. Comparison of the figures in this and my previous paper shows that the larva of *P. coarctata* is peculiar in lacking the circlets of bristles on the pointed projections and in possessing clavate adhesive tubercles on the dorsal surface of the abdomen.

The larvae are fed in the very same manner as the larvae of the large Texan Ponerinae, *i.e.*, with pieces of food and not with liquid regurgitated by the ants. In confinement I did not succeed in inducing the ants to feed their larvae with fragments of insects, but they carried crumbs of moistened corn bread to them, and the larvae could be seen lying on their backs, attached by their glutinous dorsal tubercles, slowly consuming the morsels which had been placed on their flattened ventral surfaces. The fixation of the larva to the walls of the nest seems to be an adaptation for giving freer play to the head and slender neck during feeding.

The oblong elliptical cocoons of *coarctata* are of a light buff or cream color, and vary from 2 to 3.5 mm. in length. They closely resemble the worker cocoons of *Lasius umbratus mixtus*

Nyl., var. *aphidicola*, an ant which in Massachusetts and Connecticut is often found under the same stones with the Ponera. The larger cocoons belong to the males and females, the smaller ones to the workers.

The eggs and larvae are looked after with great care by the ants, as Forel has observed.[1] On a former occasion, however, I expressed doubt concerning the validity of Forel's further statement: "Lorsqu'on découvre un nid de *Ponera* dans un tronc pourri, on voit leur cocons jaunes assemblés dans un coin, mais absolument abandonnés des ☿ qui n'essaient pas de les sauver, ni de les recueiller." I have since had frequent opportunity to observe these ants, and I am convinced that the master myrmecologist is in error. It is true that the slightest disturbance of the nest causes the ants to retreat into their galleries and to forsake their cocoons, but when one stops to watch the nest a few moments, one is sure to see the ants returning one by one and stealthily removing their charges. This they do rather awkwardly, walking backwards and dragging the cocoons away without lifting them from the ground, in marked contrast with *Leptogenys elongata*, which straddles the cocoon with its long legs and carries it away with surprising dexterity. Simple experiment with the artificial nests shows that the cocoons of *Ponera*, when removed to a distance of three or four inches from the chamber in which the ants have stored them, are taken back in the space of ten to thirty minutes. Nevertheless, Forel certainly deserves credit for directing attention to this matter of the care of the cocoons, for if one has observed the way in which a large and highly specialized ant, like our northern *Formica pallidefulva Schaufussi*, *e.g.*, when its nest is uncovered, rushes out in the very face of danger to rescue its cocoons, the slow and awkward methods of *P. coarctata* certainly indicate a more primitive or possibly degenerate condition quite in harmony with the other habits of this feeble little ant. Further evidence that these ants care for their cocoons is seen in their habit of continually creeping in and out among them, and in the time which they devote to licking and cleansing them when there are no longer any larvae to require these attentions.

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[1] *Loc. cit.*, p. 443.

Forel goes on to say of *P. coarctata*[1]: "Je soupçonne que chez ces fourmis, moins sociales que les autres, les nymphes sortent seules de leurs cocons, sans avoir besoin de l'aide des ☿." For the purpose of testing this supposition, I tried to surprise the ants in the act of leaving their cocoons, but I was not successful, notwithstanding numerous workers, males and females, were hatching in my artificial nests. A large number of cocoons from several different nests, and apparently in a healthy condition, were isolated in small dishes, but they failed to hatch. On the other hand, the workers opened many cocoons, extracted the dead or moldy pupae, cut them up into pieces, ate portions of them, and deposited the remainder on the refuse heap. I shall show when I come to consider *Stigmatomma pallipes* that these acts, which resemble what I formerly described for *Leptogenys* and regarded as indirect proof that the living callows are assisted in their escape from the cocoons, are of no value as evidence in this matter.

On hatching, the workers of *Ponera* are light dirty yellow, and very gradually, in the course of several days, acquire their dark color. The abdomen remains pale longer than the head and thorax. The females are more mature on hatching, having the head and thorax brown. The males are quite black and fully mature soon after leaving the cocoon.

In concluding this account of *P. coarctata* a few myrmecophiles that dwell with this ant may be mentioned. In two nests, one from Rockford, Ill., the other from Colebrook, Conn., I found a small brown Pselaphid beetle. In a single nest in the latter locality a minute Staphylinid was taken. In this locality also were found some peculiar mites, often attached in pairs on either side of the node and the first abdominal segment. Their symmetrical position resembles that of the mites *Antennophorus* and *Discopoma* infesting the *Lasius umbratus mixtus* Nyl. of Europe.[2] Wasmann[3] enumerates as myrmecophiles of *P. coarctata* in North America the Staphylinid beetle *Apocellus* (?) *sphaericollis* Say, and in Europe the two Pselaphid beetles: *Trichonyx sulcicollis* Rchbch and *Amauronyx Märkeli* Aubé.

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[1] *Loc. cit.*, p. 443.

[2] See Janet. Études sur les fourmis, les guêpes, et les abeilles. Note 13. Sur le Lasius mixtus l'Antennophorus Uhlmanni, etc. Pp. 1–62, 16 figs. Limoges, 1897.

[3] Kritisches Verzeichnis der myrmekophilen und termitophilen Arthropoden. Berlin, 1894.

STIGMATOMMA (AMBLYOPONE) PALLIPES HALDEMANN.

Although two species of *Stigmatomma* are known from southern Europe (*S. denticulatum* Roger and *S. impressifrons* Emery), both are of rare and local occurrence. On the former species Emery[1] has published the following note: "Dr. Alessandro Fosi had the good fortune to observe the nest of *S. denticulatum* while excavating antiquities at Verucchio near Rimini. These nests were found on several occasions in the Umbrian cinerary urns, and always at the surface of the layer of ashes with bone fragments found beneath the earth which had percolated in between the lid and the original contents of the urn. It was, however, impossible to obtain the winged individuals, nor could anything further be ascertained concerning the habits of these singular subterranean ants. The population of a nest which I had occasion to examine comprised about forty individuals, three of which were females."

Our American species, too, is considered to be an uncommon insect, although it is widely distributed through eastern North America, from Canada to North Carolina.[2] I have found it both on the Island of Naushon, near Woods Holl, Mass., and at Colebrook, Conn. In the former locality it was very common in some rather open oak woods at the north end of the island, under large stones, imbedded in rich vegetable mold. Here, on August 6, I uncovered some thirty nests in the course of three hours. At Colebrook, after the most careful search, I succeeded in finding only three nests (August 16 to 31), and these were in widely separated localities. All the nests on Naushon Island contained great numbers of cocoons, but in only three were there eggs or larvae. One of these contained a few mature larvae ready to pupate; the two others contained several packets of eggs and many young and half-grown larvae. One of the nests found in Colebrook, August 29, contained numerous callows and a few young larvae. These observations, together with the fact that the cocoons collected on Naushon Island nearly all hatched before August 20, show that *Stigmatomma* normally produces two broods during the summer. In this respect it may differ from *P. coarctata*; for, as I have said, no eggs or larvae of this species were found in several nests examined the last of August and the beginning of September.

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[1] "Sopra Alcune Formiche," etc., *loc cit.*, p. 3.

[2] Emery, "Beiträge," etc., *loc. cit.;* Forel, *loc. cit.*

S. pallipes seems to be so completely subterranean in its habits that it does not come to the surface even at night. Its nests are like those of *P. coarctata*, and it also often digs its galleries in the vegetable mold overlapping the edges instead of beneath the center of a stone. It is a much larger ant than *P. coarctata*, measuring 5.5 to 7.5 mm. One of the colonies taken on Naushon Island consisted throughout of very small individuals (5.5 to 6 mm.). The Colebrook individuals were all of smaller size than the majority of those from Massachusetts.

The females (Fig. 5), of which each colony contains from one to four before the hatching of the cocoons, are of the same size or slightly larger than the workers. They differ from the workers (Fig. 7) in having much larger lateral eyes, in having ocelli and wings, and in the structure of the thorax. Both females and workers are of a rich reddish-brown color; in older specimens the head, thorax, and node are almost or quite black, while the abdomen and legs are much paler. The male (Fig. 6) is black, with the two basal joints of the antennae, the trochanters, tibiae, and tarsi yellow; the remainder of the antennae reddish. The head, thorax, and anterior portion of the node are opaque and coarsely punctate or wrinkled, whereas the pleurae, scutellum, posterior edge of the node, and the abdomen are glabrous. The black stigma of the colorless wings in both sexes is large and conspicuous. Interesting morphological characters, such as the structure of the antennae in the two sexes, the remarkable dentate mandibles and clypeus in the female and worker, the venation, etc., are represented in the figures and need no further description. There were no ergatoid females among the many specimens collected and reared, but it is quite possible that some of the workers may lay eggs, and, though lacking ocelli, such individuals could be called ergatoid females in the sense in which I have used that term (perhaps somewhat inaccurately) in my description of *Pachycondyla harpax*.[1]

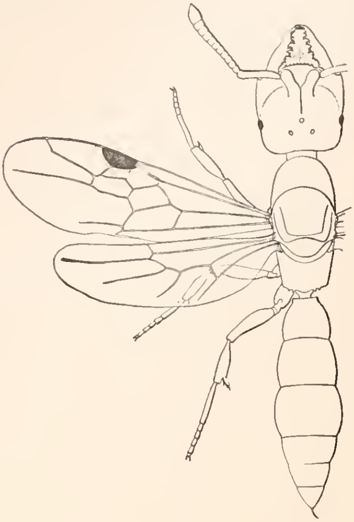

Fig. 5.

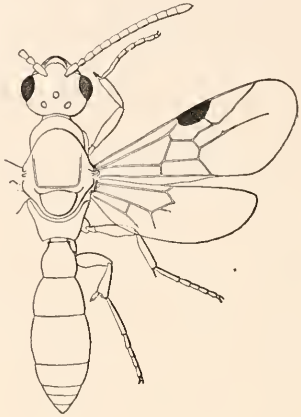

Fig. 6.

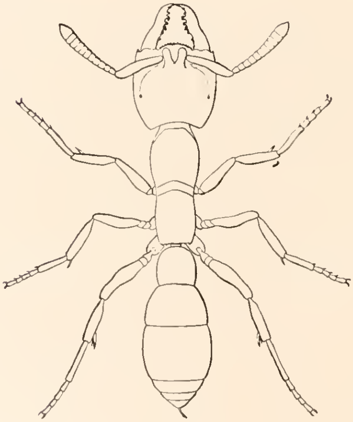

Fig. 7.

Stigmatomma pallipes Haldem. Fig. 5, Virgin female; Fig. 6, male; Fig. 7, worker.

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[1] *Loc. cit.*, p. 5.

Much of what I have said concerning the size and growth of the colonies of *Ponera* may be repeated for *Stigmatomma.* One colony taken at Colebrook contained only two workers, and another seven workers and a female; but nearly all that were collected on Naushon Island were larger, varying from ten to twenty individuals. As some of these colonies had from four to six times as many cocoons as ants, the colonies in the artificial nests by the end of August contained from forty to sixty individuals, including in some instances ten or a dozen females and as many males.

Different nests of *S. pallipes* fraternize after a struggle in much the same manner as other species of Ponerinae. When my supply of vials gave out, while collecting the numerous nests of this species on Naushon Island, I was obliged to put some fifteen nests into a single glass jar. There was considerable struggling among the ants of the different nests for a few days, but eventually they settled down peacefully and attended to their cocoons in common. Very few of the ants were killed in the struggle, and these were usually small individuals. A portion of this compounded colony was taken in a small dish on a day's railway journey, August 9. On arriving at my destination I found most of the ants killed, and a few that were still fighting died a few days later. The unusual severity of the struggle in this case was probably due to the close confinement of the ants in a small receptacle and the jarring to which they had been subjected for several hours. Four ants and a number of larvae taken at Colebrook, Conn., August 28, were placed in a Naushon nest which had just hatched its last cocoons. The larvae were at once appropriated by the Massachusetts ants, and later in the evening a sharp struggle ensued between the members of the two colonies. On the following morning one Connecticut ant was found dead, the three others had gone over to the enemy, and the whole colony was busy cleansing the larvae.

The eyes of the workers of *Stigmatomma* are even more rudimental than those of *Ponera.* The reactions to light and darkness, to contact and to moisture, closely resemble those of the above-described species. The females, notwithstanding their much larger eyes and their ocelli, have the same timid, groping habits as the workers. When fully mature the males, like the males of *Ponera*, are positively heliotropic and negatively stereotropic.

Stigmatomma appears to be very sensitive to low temperatures. It passes the cold nights and mornings even during August and September curled up, with its broad head covering the tip of its abdomen. When dug out of the soil some moments elapse before it straightens and begins to run about. It probably passes the winter in the convoluted conditions.

For fully four weeks after my colonies were placed in artificial nests the ants refused to eat, although I tried a great variety of foods. When small living insects were placed in the nest they were cautiously attacked, the ant advancing, snapping at them with its long mandibles, and then retreating. This whole action was a very feeble imitation of the snapping I have described for *Odontomachus*.[1] The insects thus killed were not eaten, but covered with particles of earth. Finally the ants consented to eat some of the larvae and pupae of *Formica pallide-fulva*, and a little later they became very fond of raw meat. While feeding, the huge mandibles are kept closed with overlapping tips. The insects are obliged to slide them over the food in order to reach it with the tongue and maxillae. Like other ants, they are unable to swallow solid food, but after rasping off a small mass with the tongue, they press it in the subpharyngeal pocket, thereby extracting its juices, and then spit out the small oblong ball of residue. The whole process of forming and disposing of these "boulettes de nettoyage" is exactly like that described by Janet for *Formica rufa*, *Lasius mixtus*, and *Vespa crabro*.[2] The workers of *Stigmatomma* were never seen feeding one another or their queens;

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[1] *Loc. cit.*, pp. 12–14.

[2] "Sur l'organe de nettoyage tibio-tarsien de Myrmica rubra L.," *Ann. Soc. Ent de France*, tome lxiii, p. 697, 1895; "Sur Vespa crabro, histoire d'un nid depuis son origine," *Mém. Soc. Zool. de France*, tome viii, p. 76, 1895; Sur le Lasius mixtus l'Antennophorus Uhlmanni, etc., pp. 16, 17. Limoges, 1897. — The agricultural ant, *Pogonomyrmex barbatus* Sm., when fed on starchy substances, like rolled oats, literally sprinkles the floor and walls of its nest with snow-white "boulettes."

nor can this be readily done by ants with such huge, horizontally projecting mandibles.

Stigmatomma has a singular habit of vigorously shaking its body from time to time, as if suffering with the ague. The motion is not unlike that of the Termites when they are supposed to be stridulating. It is possible that the action of the Ponerine may produce a sound through the rubbing of the various segments and joints on one another, but, though perfectly familiar with the sounds produced by the large Myrmicines *Pogonomyrmex* and *Atta*, I have not been able to detect them in *Stigmatomma*.

What I have said concerning the habits of cleanliness in *P. coarctata* applies also to the species under consideration.

The eggs of *Stigmatomma*, though deposited by a larger insect, are smaller than those of *P. coarctata*, being only .5 mm. long. They are, however, more numerous. A packet deposited by a female in one of my artificial nests contained thirteen eggs. In shape they are oblong elliptical, like the eggs of other Ponerinae. They are not arranged with their long axes parallel with one another, as in *Pachycondyla* and *Leptogenys*, but in an irregular mass, like the eggs of all the other subfamilies of ants, including the Dorylinae (*Eciton*).

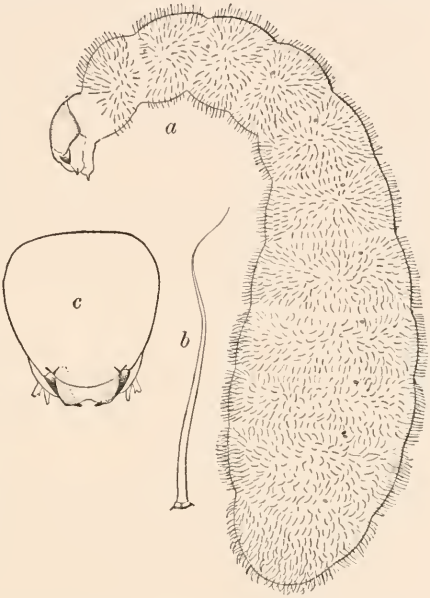

Fig. 8. — *a*, mature larva of *Stigmatomma pallipes* Haldem; *b*, bristle of same; *c*, head of same (dorsal aspect).

The larva has the appearance of Fig. 8, *a*. The body is rather slender in alcoholic specimens, and the segments are all quite distinct and clothed rather uniformly and densely with yellowish hairs, which under a high power (Fig. 8, *b*) are seen to taper into very slender flexuous points. The head is somewhat longer than broad and without hairs on its dorsal surface, the labrum is bilobed, the maxillae provided with the usual tactile cones. The outer one of these on either side appears to be bifurcate. The young differs from the mature larva only in having a relatively larger head and a sparser covering of bristles. Comparison of the larva of *Stigmatomma* with that of *Ponera*, *Pachycondyla*, *Leptogenys*, and *Odontomachus* shows that it does not conform to the Ponerine type but closely resembles, instead, the larvae of certain Myrmicinae, which are also covered with hairs instead of bristly tubercles.[1]

The cocoons of *Stigmatomma* are of a slightly darker, more brownish color, and somewhat more oval than the cocoons of *Ponera*. They measure from 4.5 to 6 mm. in length.

What I have said of the care of the eggs, larvae, and pupae of *P. coarctata* is equally true of *Stigmatomma*.

The larvae are fed in the very same manner as other Ponerine larvae. In one of the nests on Naushon Island a large larva was seen with its head and neck inserted in the two last segments of a beetle larva (*Tenebrionid*), which must have been captured and disarticulated by the ants. In my artificial nests the ants carried the larvae of *Formica pallide-fulva* to their own young. The latter could be distinctly seen sucking the juices of the *Formica* larvae till they were reduced to shriveled skins. These were then carried away and placed on the refuse heap.

I have succeeded in surprising the callows in the act of escaping from their cocoons. This they do, as Forel believed to be the case with *P. coarctata*, without any assistance from the workers. Several cocoons were isolated in a watch-glass, and I had an opportunity of seeing a female, two males, and several workers emerge entirely by their own efforts. The ant gnaws through the wall of the cocoon at a spot a short distance behind the anterior pole. The shape of the incision at once indicates whether a male or a female (or worker) is about to emerge. In the latter case the opening, which is produced by the huge mandibles, has the form of a transverse slit, extending halfway round the cocoon. The small, sharp mandibles of the male, however, gnaw a hole with irregular edges and of much smaller size. The insect, after periods of struggling, alternating with periods of rest, succeeds in getting first one antenna, then the other, and then the fore legs through the orifice, and finally, with considerable effort, creeps out. After making this observation on isolated cocoons I had an opportunity of making it in the artificial nests. In these the hatching cocoons were often carried about and placed on or under the stack of other cocoons, while the callows, struggling to emerge, seemed to hold out their antennae and fore legs in a supplicating attitude to the completely indifferent workers. In a few instances the callows died while halfway out of the cocoons and were carried to the refuse heap in this condition. Occasionally, when the young callows had emerged with their hind legs still enswathed and encumbered by the white pupal skin, the workers would pull this away. They also occasionally licked and fondled the newcomers, as if their bodies were covered with some pleasant secretion, but beyond these acts their helpfulness did not extend. These same workers, however, frequently opened cocoons and extracted dead immature pupae, cut them up, and then placed them on the refuse heap. This act shows that the statements concerning *Leptogenys* in my former paper[1] may require emendation.

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[1] Compare, *e.g.*, my figures of the larvae of *Pogonomyrmex barbatus*, *loc. cit.*, p. 20, Fig. 9.

The newly hatched *Stigmatomma*, as we should naturally expect from the above observations, is not as feeble as the callows of the more specialized ants. The males and females issue with their wings fully expanded; the former have their bodies completely pigmented and are able to run about briskly; the latter, as well as the worker callows, although of a rich yellowish-red, a color which they retain for several days, are nevertheless soon able to run about and to join in the labors of the colony. The queens show no tendency to leave the nest and usually lose their wings (after copulation ?) while still in the red callow condition.

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[1] *Loc. cit.*, pp. 29, 30.

Careful search in and about the natural nests of *Stigmatomma* failed to reveal the presence of any myrmecophiles. Wasmann, however, mentions [1] a Pselaphid beetle, *Araniops amblyoponica* Brend., as occurring with these ants in Pennsylvania and North Carolina.

GENERAL CONSIDERATIONS.

The foregoing observations on *Ponera* and *Stigmatomma*, together with those contained in my former paper, suggest some considerations of a more general nature.

First, the appearance of the larva of *Stigmatomma*, differing so widely from the known larvae of other Ponerinae, is calculated to raise some doubts on the subject of taxonomy. Forel [2] has been of the opinion that the group of genera including and allied to *Amblyopone*, viz., *Stigmatomma*, *Mystrium*, *Prionopelta*, and *Myopopone*, should be separated from the Ponerinae as an independent subfamily, the Amblyoponinae. The main characters used as an argument for this change are the presence of two spurs on the hind tibia of these ants, and the very broad and peculiar articulation of the node with the succeeding abdominal segment. Emery, on the other hand,[3] has protested against raising this group to the rank of a subfamily and its separation from the Ponerinae.[4] He argues that double spurs occur also on the hind tibiae of nearly all Ponerinae and of several Myrmicinae as well, that the articulation of the node is highly variable among the Ponerinae, and that the Cerapachyi (which Emery insists on placing with the Dorylinae) present the same conditions of the node as in *Amblyopone*. He also calls attention [5] to the important fact that the male genitalia of *Stigmatomma serratum* Hald. are constructed "soweit es ohne Zergliederung zu sehen ist, ganz nach dem gewöhnlichen Ponerinentypus."

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[1] *Loc. cit.*, p. 94.

[2] "Sur la classification de la famille des Formicides," *Annales de la. Soc. Entomol. de Belgique*. Tome xxxvii, pp. 161–165. 1893.

[3] "Die Gattung Dorylus Fab. und die systematische Eintheilung der Formiciden," *Zool. Jahrb.* Abth. f. System. Bd. viii, pp. 685-778, Taf. XIV–XVII, 41 text-figs. 1895.

[4] "Die Abtheilung der Amblyoponinae darf nach meiner Ansicht nur als Tribus in der Subfamilie der Ponerinen beibehalten werden."

[5] *Loc. cit.*, p. 694, footnote.

It would seem, therefore, that there are no very cogent reasons for adopting the subfamily Amblyoponinae, so far as characters drawn from the adult structure are concerned. The habits of *Stigmatomma,* as I have shown, are essentially the same as those of the Ponerinae, so that there exist no ecological grounds for accepting Forel's suggestion. The larva, however, seems to me to show very clearly that there is a greater gap between the Amblyoponii as a tribe of Ponerinae, and the tribes Ponerii and Odontomachii, than between the two last-mentioned groups. It must be remembered, however, that the larvae of two tribes of Ponerinae, the Australian Myrmecii and the cosmopolitan Ectatommii, have not been described, and that when these are known the striking differences between the Amblyoponii and the Ponerii may be reconciled. If, as Emery suggests,[1] the Myrmicinae are descended from the Ponerinae, it is obvious from a study of the larvae that the former subfamily must have come from forms with larvae like the Amblyoponii, a group which in other respects also is generally regarded as very primitive.[2] For the present I can see no reasons for altering the excellent classification outlined by Emery in his *Dorylus* paper.

There still exists an ecological (or biological, in the German sense) connection between the Ponerinae and the Myrmicinae, as I have lately ascertained. Since describing the peculiar method employed by the Texan Ponerinae in feeding their larvae, I have found that one of our New England Myrmicine ants, *Stenamma* (*Aphaenogaster*) *fulvum* Rog., subsp. *aquia* Buckl., var. *piceum* Emery — an ant very common under stones and in rotten logs along the edges of woods — has essentially the same method of feeding its young ! My attention was first called to this fact in an artificial nest belonging to Miss Adele M. Fielde, at Woods Holl, Mass. One afternoon Miss Fielde left a lot of queen pupae and larvae of *Crematogaster lineolata* within reach of the *Stenamma* colony. By the following morning the Stenammas had carried these into their nest, cut off their heads and abdomens, and had distributed the pieces freely among the larvae, which could be seen singly and in groups of from two to five eagerly feeding on the juices in the same manner as Ponerine larvae. Thinking that this might be a very exceptional action, due to the confinement of the colony, I opened numerous nests in the woods during the month of August, while the ants were rearing their second brood. In nearly every one of these nests I found one or more larvae feeding on substances left among them by the workers. In one nest three larvae were feeding on a small Geometrid caterpillar; in another several had their heads and necks inserted into the thoraces of some small Carabid beetles that had been decapitated by the ants; in still another nest several larvae were devouring the pulp of a blackberry, etc.[1]

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[1] *Loc. cit.*, p. 773. "Dagegen liefern die Ponerinen offenbar die Wurzel, aus welcher die übrigen Unterfamilien der Ameisen (*i.e.*, after excluding the very primitive Dorylinae !) d. h. die Myrmecinen, Dolichoderinen und Camponotinen entsprossen sind."

[2] Emery, *loc. cit.*, p. 774, suggests that the Myrmicinae may be related to the Ectatommii, while the Dolichoderinae are more closely allied to the Ponerii.

Since making the above observation I find that Janet has recorded some very similar facts.[2] He saw several large larvae of *Lasius mixtus* and *L. flavus* sucking the juices from the cadavers of small larvae of their own species. Another more detailed observation I quote *in extenso:* "C'est dans un nid artificiel de *Tetramorium caespitum* que j'ai pu faire, à ce sujet, l'observation la plus précise. Au moment où les Fourmis récoltées venaient de terminer leur emménagement, j'ai vu, de la façon la plus nette, une larve d'ouvrière sucer une petite larve jaune de Coléoptère. La larve de *Tetramorium* n'était pas très éloignée d'avoir atteint sa taille définitive. Elle était suspendue par ses poils d'accrochage contre la paroi du nid, immédiatement sous le plafond en verre. Elle était placée horizontalement, le dos en haut, mais un peu de côté.

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[1] *Stenamma fulvum* also brings into its chambers numerous seeds and the corollas of small white flowers. I mention this fact because it shows that this ant, which is normally carnivorous, nevertheless has proclivities that ally it by instinct as well as by structure to the harvesting ants of the subgenus *Messor*.

[2] Le *Lasius mixtus*, etc., *loc. cit.*, pp. 10–12.

Au-dessous d'elle, placée tête-bêche, parallèlement à son corps et soutenue en partie par les poils d'accrochage de l'abdomen du *Tetramorium*, se trouvait la petite larve jaune vermiforme, ayant ½ millimètre de diamètre et 2 millimètres ½ de longeur. Cette larve jaune avait certainement été placée là par une ouvrière, car pendant l'emménagement, j'en avais vu une qui introduisait une larve semblable dans le nid. La larve de *Tetramorium* avait sa tête infléchie et appliquée contre la larve jaune. Elle laissait voir, très nettement, sa bouche et ses pièces buccales. Grâces à ces circonstances exceptionnellement favorables j'ai pu examiner, avec une forte loupe, ce qui s'est passé, et cela pendant plus d'un quart d'heure. J'ai d'abord constaté le mouvement incessant de la bouche et vu nettement l'absorption du liquide transparent qui sortait de la plaie. Libre dans sa partie moyenne, la petite larve jaune était soutenue dans sa région céphalique par les poils d'accrochage de l'abdomen du *Tetramorium*. Ce dernier maintenait, au moyen de ses mandibules crochues, l'extrémité anale de sa proie, et cette extrémité était animée d'un mouvement rhythmé de balancement résultant des mouvements de succion. Pendant ce repas, et sans que la larve du *Tetramorium* parut en être dérangée, une ouvrière est venue la lécher. Cette ouvrière est allée, ensuite, dégorger de la nourriture contre la bouche d'une larve voisine. Au bout d'un quart d'heure j'ai du interrompre l'observation parce qu'une ouvrière est venue, malencontreusement, intercaler une nymphe entre la larve et le verre. J'ai alors pris la larve avec un pinceau et une petite cuiller et j'ai constaté qu'elle avait ramené sa bouche contre son corps, et que le repas était interrompu. Quant à la petite larve jaune dont j'avais vu le corps bien gonflé au commencement de l'observation, elle était, maintenant, surtout dans la région sucée, flasque et en partie vidée." Janet concludes his observations on this subject with the words: "Cette observation, venant confirmer celles, un peu moins précises, que j'ai faites chez d'autres espèces, ne me laisse plus aucun doute sur la faculté que les larves de Fourmis possèdent de sucer directement les liquides contenus dans le corps de larves, probablement blessées au préalable, qui les ouvrières deposent auprès

d'elles. Toutefois, cette manière de prendre la nourriture doit être considerée comme tout à fait exceptionelle."

The fact that this peculiar method of feeding the larvae is the only method adopted by the Ponerinae, and, I believe, also by the Myrmicine *Stenamma fulvum*, and that it occurs as an exception in such highly specialized ants as *Lasius* and *Tetramorium*, is of considerable interest from the standpoint of the phylogeny of instincts. It not only spans a gap between the generalized Ponerinae and the more specialized Formicinae, but it would seem to indicate that the method of feeding the larvae by regurgitation was grafted on to this original method in the more recent ants, possibly in connection with their habit of feeding one another by regurgitation in their adult conditions.[1]

In conclusion, the various peculiarities which indicate that the Ponerinae are a very primitive and generalized subfamily of ants may be enumerated[2]:

1. The colonies of the Ponerinae consist of a comparatively small number of ants, like the incipient colonies of the Myrmicinae, Dolichoderinae, and Formicinae.

2. These small colonies appear to be annual growths, formed by swarming, as in the bees, and not by single fertilized female ants unaccompanied by workers, as in the above-mentioned subfamilies.

3. Two or more colonies of Ponerinae of the same species can be fused to form a larger colony without much difficulty. This is not so easily accomplished with many species of the more specialized ants.

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[1] Since completing my manuscript I find that one of our Texan Myrmicine ants (*Pheidole* sp. near *P. fabricator* Smith) resembles *Stenamma fulvum* in its manner of feeding the larvae. September 27 I opened a small nest of the *Pheidole* near Austin and found dozens of larvae feeding on fragments of different insects collected and comminuted by the workers. This is of interest because the *Pheidole*, like its congeners, is a harvesting ant, storing the large flat chambers of its nest with many seeds.

[2] While I have assumed in this and in my former paper that the Ponerinae may be regarded as the group from which the Myrmicinae, Dolichoderinae, and Formicinae (Camponotinae) have developed, I am quite of Emery's opinion that the existing Ponerinae are not ancestral forms. Emery calls attention to the fact that the palpi are aborted in all the tribes of Ponerinae except the Myrmecii, whereas many of the genera of higher ants have retained the undiminished number of joints in these organs.

4. The architecture of the Ponerinae is of a primitive character, consisting of a few irregular and unfinished galleries and chambers. The latter are not even formed by all the species.

5. The queen and worker differ but little in size and structure.

6. Ergatoid females, or forms intermediate between the queens and workers, are of normal and comparatively frequent occurrence in some species.

7. The habits of the queen and worker are very similar. The female is not an individual on whom special attention is bestowed by the workers.

8. The workers show no tendency to differentiate into major and minor castes.

9. The Ponerinae are carnivorous and live by hunting (in contrast with the various harvesting, fungus-growing, honey-collecting, and aphidicolous members of the more specialized subfamilies).

10. They do not feed one another by regurgitation.

11. The larvae are not fed by regurgitation, but are given pieces of insects from which they suck the juices.

12. The cocoon is retained as a pupal envelope throughout the group. (The Ecitonii among the Dorylinae have lost this envelope, although the Dorylii still retain it; it is lost in the Myrmicinae, and is apparently in the process of disappearing among the Formicinae.)

13. In at least one genus (*Stigmatomma*) the callows are able to escape from their cocoons without the assistance of the workers.

14. The callows of the Ponerinae are more mature on leaving the cocoon than the newly hatched Formicinae.

Colebrook, Conn., Sept. 5, 1900.

FUSION OF FILAMENTS IN THE LAMELLIBRANCH GILL.

EDWARD L. RICE.

It is far from the purpose of this paper to enter fully into the discussion of the morphology of the lamellibranch gill; but a brief preliminary statement of a general nature may serve to define the terminology employed, and to simplify the following more detailed description of a single point in the gill structure.

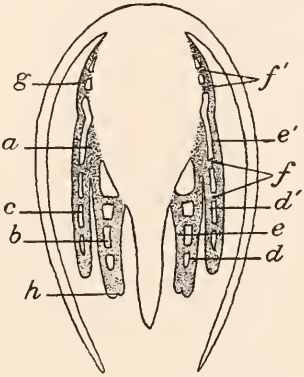

Fig. 1. — Diagrammatic frontal section of lamellibranch. Shell omitted; gills shaded. *a*, gill axis; *b*, inner gill; *c*, outer gill; *d* and *d'*, descending lamellae of inner and outer gills; *e* and *e'*, ascending lamellae of inner and outer gills; *f* and *f'*, interlamellar connections of gill and dorsal appendage; g, dorsal appendage of outer gill.

When the animal is oriented in the usual manner, with the hinge line upward, the gills hang down on either side, between the body and mantle, as shown in Fig. 1. Strictly considered, there is but one gill, or one ctenidium, on each side of the body. This ctenidium consists, fundamentally, of a slight longitudinal ridge along the side of the body, the gill axis (Fig. 1, *a*), and a double row of ciliated filaments, which extend downward into the mantle cavity of the animal and are then reflected upward. In common language each of these rows of filaments, or the structure arising therefrom, is termed a gill; and I shall retain this convenient, though morphologically indefensible term, and designate that half of the ctenidium next the body as the inner gill (Fig. 1, *b*), and that half next the mantle as the outer gill (Fig. 1, *c*).

In some few forms the adjacent gill filaments remain entirely free from one another, *e.g.*, Anomia; in other forms, as Mytilus and Pecten, there is a union by means of tufts of very long interlocking cilia — ciliated discs. In contrast to these two types, which may be grouped together as filamentous gills, we find in the great majority of lamellibranchs a more or less strong development of vascular connections between the filaments, binding them together to form the lamellae from which the class takes its name. Such gills may be termed lamellar.

As each filament consists of two limbs, a descending and an ascending, so each gill is composed of two lamellae, correspondingly designated as descending (Fig. 1, *d* and *d′*) and ascending (Fig. 1, *e* and *e′*). These lamellae are usually connected with one another by more or less complicated interlamellar connections (Fig. 1, *f*).

The ascending lamella of the inner gill may remain free, or its upper margin may fuse with the body and, behind the body, with the corresponding part of the gill of the other side. The ascending lamella of the outer gill may lie free in the mantle cavity or may be attached to the mantle on a level with the gill axis; or it may be continued dorsally above this line, forming a dorsal appendage (Fig. 1, *g*), which is finally attached to the body, or rather to the fusion line of mantle and body. Thus the dorsal appendage consists of the ascending lamella alone, and may show a structure decidedly different from that of the gill proper. Connections similar to the interlamellar connections of the gill attach the dorsal appendage to the body wall (Fig. 1, *f′*).

The lower free margin of some gills is smoothly rounded off; in other cases the border is deeply notched by a groove running from end to end of the gill. This may be called the marginal groove. In the diagram the groove is seen in cross-section in the inner gill (Fig. 1, *h*), while the outer gill presents a smooth margin with no sign of a furrow. Exactly these conditions are found in a large number of lamellibranchs, *e.g.*, Astarte, Dreissensia, Cardium, Psammobia. It may be suggested in passing that the somewhat unintelligible distinction of "double gills" and "single gills," in the classical paper by Williams,[1] may perhaps be based on the presence or absence of this marginal groove.

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[1] Williams, T., "Respiratory Organs of Invertebrates," *Annals and Magazine of Natural History*. Vol. xiv. 1854.

The surface of the gill lamellae may show either of two types. It may be smooth, all the filaments lying in one plane; or, with the requirement of greater respiratory surface, the lamellae may be folded parallel to the filaments. The two lamellae of one gill are always folded symmetrically, so that the section of the gill perpendicular to the filaments assumes an outline reminding one, in its extreme form, of a string of wooden button molds. Both filamentous and lamellar gills are subject to this folding.

The filaments occupying the bottom of the reëntrant folds, the limiting filaments, are usually somewhat larger than the intermediate filaments, and are often very much modified in form as well as size. These filaments may be easily traced through the whole height of the gill and afford a series of fixed points, aiding materially in the study of the folded type of gill by means of sections.

From the above résumé it is evident that fusion or concrescence of parts has long been recognized in the lamellibranch gill. Even in simple filamentous gills (Mytilus, etc.) the tips of the filaments fuse to form a continuous band along the margin of the ascending lamella. In more complex forms it is a process of fusion which transforms the rows of originally distinct filaments into the characteristic lamellae. The fusion of the ascending lamellae with the mantle and body is also perfectly familiar. But the particular type of fusion described below appears to have escaped mention in the somewhat extensive literature upon the lamellibranch gill.

Even a hasty study of serial sections of the strongly folded gill of *Cardium edule* or *Batissa tenebrosa* shows the somewhat surprising fact that each fold contains a far larger number of filaments in the upper portion of the gill than in the neighborhood of the free margin, this being equally true of ascending and descending lamellae. (The extreme case was noted in Cardium, where the number of filaments in one fold increased from eight to thirty.) Yet the limiting filaments are continuous throughout, showing that there is no reduction in the number of folds correlated with the increase in the number of filaments in each fold. These observations appear at first sight irreconcilable with the theory of gill development advanced by Lacaze-Duthiers[1] and universally accepted, according to which the ascending limbs of the filaments are essentially the reflected tips of the originally simple straight filaments. The larger number of filaments in the upper parts of the descending lamellae could be explained by the assumption of the presence of a large number of abortive filaments. But this explanation, unsatisfactory even for the descending lamellae, cannot apply to the ascending lamellae.

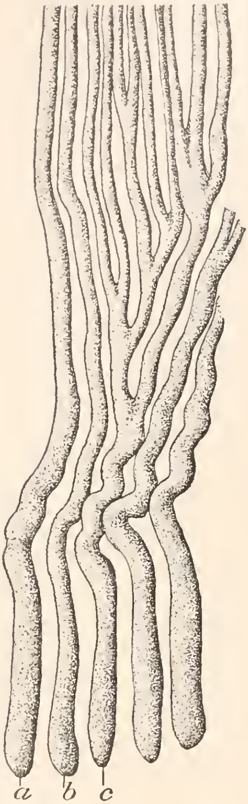

FIG. 2. — Surface view of group of filaments of inner gill of *Batissa tenebrosa*. × 60. Lower edge of figure represents the free margin of gill. Cilia and complex interfilamentary connections omitted for the sake of clearness. *a* and *b*, simple filaments; *c*, compound filament, formed by fusion of nine simple filaments.

A more detailed study of serial sections, and, better, of microscopic dissections in which the lamellae are separated and the folds spread out as smooth as possible, shows clearly that the filaments of the upper portion gradually meet and fuse as the free margin of the gill is approached. This phenomenon is illustrated by Figs. 2 and 3. In the former we have a surface view of a small portion of the inner gill of *Batissa tenebrosa*, extending upward from the free edge. While the two filaments at the left (Fig. 2, *a* and *b*) are simple throughout, the next filament (Fig. 2, *c*), indistinguishable from the others at its lower end, is really formed by the fusion of nine filaments.

In this species such groups of fusing filaments are found principally in the projecting folds of the gill, very seldom in the reëntrant folds. In *Cardium edule*, on the other hand, the fusion is more marked in the reëntrant than in the projecting folds.

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[1] Lacaze-Duthiers, H. de, "Mémoir sur le développement des branchies des Mollusques Acéphales Lamellibranches," *Annales des Sciences Naturelles.* Zoologie Sér. iv, Tome v. 1856.

Fig. 3 represents diagrammatically the surface of a single fold of this species, each filament being represented by a simple line. At the gill margin (lower side of diagram) there are eight filaments, including the limiting filaments (Fig. 3, *a* and *h*); in the upper part of the gill (and diagram) there are thirty. Of these thirty filaments, twenty-one unite to form the two limiting filaments (Fig. 3, *a* and *h*) and the two adjacent intermediate filaments (Fig. 3, *b* and *g*), which occupy the re-entrant folds. The remaining four intermediate filaments (Fig. 3, *c*, *d*, *e*, and *f*), those of the projecting fold, are formed by the union of only nine filaments. The irregularity of the fusion is also well shown by this diagram. Note especially that one limiting filament is entirely simple, while the other is the resultant of the fusion of seven simple filaments.

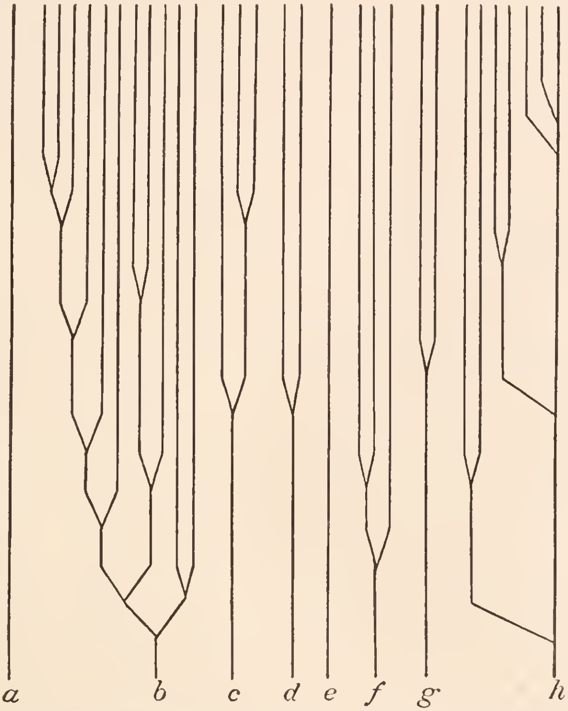

Fig. 3. — Diagrammatic view of surface of fold of inner gill of *Cardium edule*, illustrating fusion of filaments. Filaments represented by lines. Lower edge of diagram represents free margin of gill. *a* and *h*, limiting filaments; *b*–*g*, intermediate filaments.

The fusion is usually almost exclusively limited to a somewhat narrow zone in the near vicinity of the free margin of the gill. In this zone may also be noted a gradual reduction in the folding of the gill as the margin is neared. In those lamellibranchs whose outer gill is provided with a dorsal appendage, there is a second zone of fusion along the transition line from the gill proper to the appendage. This line again marks a partial or total obliteration of the folding of the gill — a point of importance in the consideration of the meaning and cause of the fusion of the filaments.

The term "fusion of filaments" has been employed repeatedly; but the question arises whether the phenomenon under discussion is really a fusion of a large number of once distinct filaments or a branching of a relatively small number of original filaments; whether, in other words, the primary filaments are represented by the maximum or minimum number. The answer is not far to seek. At the free margin of the gill the continuity of the filaments may be readily traced from one lamella to the other, and the number of filaments in the two lamellae within any given fold must necessarily be equal. A little higher, in the zone of fusion, these numbers may become very unequal. For example, in a section of the gill of Batissa seven filaments of one lamella were observed to correspond to twelve in the other. Neither of these facts, however, offers conclusive evidence; both may be explained as well on the supposition of branching as on that of fusion. But as the serial sections are followed a little higher in the gill, the inequality in the number of filaments, which has increased from zero at the margin to a maximum in the zone of fusion, begins to fall off again more or less rapidly, and finally reduces itself once more to zero. This equality in the number of filaments in the upper parts of the gill can be explained only on the supposition that the phenomenon before us is a fusion, not a branching, of the gill filaments. It is altogether too improbable that the independent branching of the filaments in the two lamellae would lead to the same number in the two cases.

Granted that the phenomenon is a fusion, what is its meaning? Is it of systematic importance? My observations are not complete enough to permit a categoric answer; but I am strongly inclined to the belief that the fusion carries no more of weight from the systematic standpoint than does the folding of the gill.

Among smooth gills no fusion was observed, although a considerable number of forms were studied. Especial attention was devoted to Tellina and Scrobicularia, in which, for reasons detailed in an earlier paper,[1] I consider the simplicity and smooth surface of the gill to be secondary characters — a retrograde development from the folded gill type of the Veneridae. Fusion was also never observed in the filamentous type of gill.

The results of my own observations upon the folded lamellar gills may be tabulated as follows:

1. Fusion strongly developed: *Cardium edule* Lin., *Chama pellucida* Brod., *Batissa tenebrosa* Hinds, *Psammobia vespertina* Lin., *Donax serra* Chemn.
2. Fusion moderately developed: *Venus verrucosa* Lin., *Cyprina islandica* Lin., in latter strongly developed on transition line from outer gill to appendage.
3. Fusion very slightly developed: *Ensatella americana* Verrill, *Mya arenaria* Lin., *Donax politus* Poli, in latter perhaps accidental.
4. No fusion observed: *Cytherea chione* Lin., *Donax trunculus* Lin., *Ostrea virginiana* Lister, *Thracia papyracea* Poli.

Lima and Ostrea should probably be added to the list of forms in which fusion occurs, though on somewhat doubtful evidence, which will be mentioned later.

Thus we find this fusion of filaments similarly developed in widely separated forms, belonging to very diverse groups; on the other hand, within the single genus Donax we find all grades of fusion, as we also find all grades of folding.

This parallelism of folding and fusion in the genus Donax appears to me to furnish, in a certain sense, an epitome of the whole matter, for I consider the fusion of the filaments to be a mechanical correlative of the folding of the lamellae. The process may be pictured in something this way. The folding of the lamellae is gradually developed in the young lamellibranch as the increasing number of filaments becomes too great to lie in one plane. But at the free margin the filaments are bound somewhat firmly together and the folding is somewhat reduced. This leads to a crowding together of the filaments at this point and eventually to an organic fusion of the same. How great the crowding must really be in the strongly folded forms may be inferred from the fact that the upper part of the inner gill of

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[1] "Die systematische Verwertbarkeit der Kiemen bei den Lamellibranchiaten," *Jenaische Zeitschrift für Naturwissenschaft.* Bd. xxxi. 1897.

Cardium edule would measure, if flattened out, fully seven times the length of the free margin of the same gill. The absence of fusion in filamentous gills, even where the folding is extreme, as in Pecten, may be easily explained on the ground of the looser structure of the gill and the possibility of a displacement of the filaments, with consequent relief of pressure.

It is an interesting point in this connection that no fusion was observed in the outer gill of either Psammobia or Cardium, although it is conspicuous in the inner gill. In both cases the inner gill is provided with a deep marginal groove, while the outer gill shows no sign of this structure. The presence of this marginal groove is the mechanical equivalent of a shortening (slight to be sure) of the margin of the gill, the bottom of this groove being almost perfectly straight, and therefore slightly shorter than the somewhat scalloped margin of the folded gills in which no groove is present. Hence we find in this characteristic a cause of increased crowding and increased fusion.

For a very short distance above each point of fusion one observes a slight modification of the epithelium of those sides of the fusing filaments which are turned toward each other. The nuclei are somewhat larger and more closely crowded, as shown diagrammatically in Fig. 5. Aside from this, no histological distinction can be drawn between the simple original filaments of the upper portion of the gill and the compound filaments of the lower margin. Even in the matter of size there is no noticeable difference except in the immediate vicinity of the fusion, where the compound filament is considerably enlarged. This statement is made after comparison of a large number of preparations, and seems to be the rule, although there are considerable individual variations in different specimens. The apparent larger size of the compound filaments in Fig. 2 is to be explained in part on the ground of such individual variations. It is due in larger degree, however, to slight differences in the position of the filaments, which are elliptical in cross-section, and appear of different size according as one or another side is presented to view. It should also be noted that the filaments are somewhat enlarged at the extreme margin of the gill, and that these enlarged tips are shown in the figure.

As regards the size and finer structure of the filaments, the evidence of sections is more reliable than that of surface preparations; and in Figs. 4–8 are represented a series of sections through a group of filaments of the inner gill of *Cardium edule.* The same filaments are shown in all the figures, although the very complex interfilamentary and interlamellar connections, differently cut in the different sections, cause a considerable variety of aspect. These connections may be disregarded in the present discussion. In Fig. 4, which represents the uppermost section, six filaments are shown, all practically alike. In Fig. 5 two of these, marked with an asterisk in all the figures, show a slight modification of the epithelium, as described above. This section is immediately above the point of fusion of the two filaments. Fig. 6 shows these filaments so closely approximated that they may almost be described as forming a single deeply grooved filament, a condition better shown by other preparations. In Fig. 7 the fusion is complete, but the compound filament is still considerably enlarged; while in Fig. 8 the compound filament has regained its normal size and form, and the transition is complete.

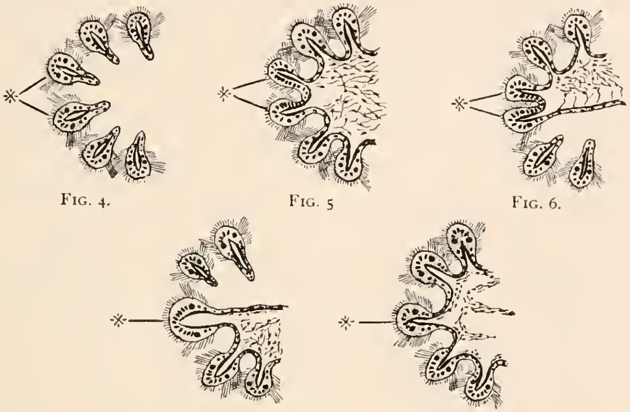

Fig. 4. Fig. 5 Fig. 6.

Fig. 7. Fig. 8.

Figs. 4–8. — Series of sections through group of filaments of inner gill of *Cardium edule.* × 95. Cilia and nuclei somewhat diagrammatic. Fig. 4 is uppermost section. Asterisk marks two simple filaments fusing to one compound.

Strangely, this very conspicuous fusion of the filaments appears to have received little or no notice. I have been able to find no reference whatever in the text of any articles at my disposal. Only in the figures is a possible hint of its observation. Thus in the exquisite work by Deshayes[1] on the Mollusca of Algiers there is a small figure of the gill of Pholas, in which two incomplete filaments are interpolated among those which extend through the whole height of the gill. In the one the free end is turned upward; in the other, downward. Do these represent two filaments fusing with their neighbors? If so, the difference in direction in the two cases clearly distinguishes the phenomenon from that described above. Moreover, the author's description of the gill as entirely smooth makes a fusion *a priori* improbable. An inaccuracy in the drawing, which is very small, is the simpler explanation.

In a considerable number of more recent figures a single fold of the gill is represented in section as containing an unequal number of filaments in the two lamellae, thus in Cardium (van Haren),[2] Lima (Pelseneer),[3] *Donax trunculus* (Sluiter),[4] and Ostrea (Kellogg).[5] It will be noted that in the last two cases the figures cited do not accord with my own observations. But my purely negative verdict of "not observed" contains no direct contradiction of the affirmative statements of these authors. While I am convinced that my own preparations show no evidence of a fusion of filaments in Ostrea and *Donax trunculus*, I consider it probable that the figures of Kellogg and Sluiter, as well as those of van Haren and Pelseneer, point to a greater or less development of the phenomenon described. Unfortunately no certain conclusions can be reached in the absence of corroboration in the accompanying text.

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[1] Deshayes, G. P., "Exploration scientifique de l'Algérie," *Zoologie*. Tome i, No. 4. Paris, 1849.

[2] Haren-Noman, D. van, "Die Lamellibranchiaten, gesammelt während der Fahrten des Willem Barents, 1878 and 1879," *Niederländisches Archiv für Zoologie*. Supplementband I, 1881–82.

[3] Pelseneer, P., "Contribution à l'étude des Lamellibranches," *Archives de Biologie*. Tome xi. 1891.

[4] Sluiter, C. P., "Beiträge zur Kenntniss des Baues der Keimen bei den Lamellibranchiaten," *Niederländisches Archiv für Zoologie*. Bd. iv. 1878.

[5] Kellogg, J. L., "A Contribution to our Knowledge of the Morphology of the Lamellibranchiate Mollusks," *Bulletin of the U. S. Fish Commission*. Vol. x. 1890.

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PORTABLE ANT NESTS.

ADELE M. FIELDE.

In order to keep ants under continued observation, and at the same time to change occasionally the domicile of the observer, it is necessary to have portable nests. The transportation of either the Lubbock or the Janet nest is made inconvenient by the water used for the isolation of the ants in the former, and by the considerable weight of the latter.

Six colonies can be successfully carried on journeys of several hours or days by the use of a case made of half-inch pine boards and dovetailed at the corners, with a door hinged upon its lower side and held shut by two buttons at its upper edge. The case measures on the inside 16¾ inches in length, 6¼ inches in horizontal depth, and 4¾ inches in height. Three shelves, each one-fourth of an inch thick, are mortised into the ends of the case, making four compartments, each one inch high. Several holes are bored in the door of the case and in the side opposite the door, to admit fresh air. The case is carried by a leather handle fastened lengthwise to its top, and it is just filled by the six nests hereinafter described.

Of the six nests, there are two of each pattern, A, B, and C. An A and a B nest together fill a shelf, while a C nest fills a shelf alone.

The nests are all built of clear glass, and their parts are readily cut by any glazier. The joinings are made with Le Page's liquid glue, which must thoroughly dry before the nests are used. The cost of material and the amount of labor required in making these nests are much less than for either the Lubbock or the Janet pattern.

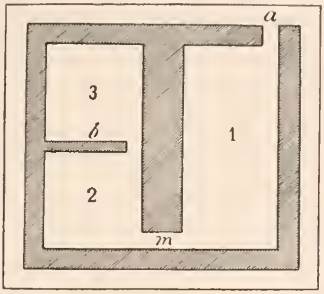
The floors are panes of "double thick" glass, under which is laid a thick sheet of white blotting paper of the same size as the glass, to give opacity, elasticity, and a background against which the ants are easily seen. The blotting paper should not be fastened to the glass, as the latter must sometimes be raised in order to get a view of the ants from below.

The walls stand half an inch from the edges of the base-pane, for the greater security of the superstructure when the nest is being lifted. The walls are built of strips of "double thick" glass, half an inch wide, two horizontal layers in every wall giving a perfectly level top. Apertures three-eighths of an inch wide are left in the walls at points marked *a* on the plans. These apertures admit the end of a three-eighths-inch glass tube, which may be used as a safe bridge between nests when ants are to be made to pass from one nest into another. When not in use, the apertures are closed with plugs of cotton. The outside of the walls, after thorough drying, are painted black, to secure within the nest the darkness in which ants like to keep their young.

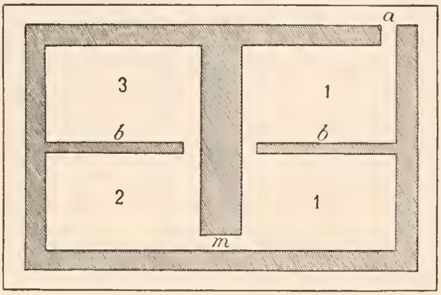
The main partitions in the ant-dwelling take part with the walls in the support of separate roof-panes, and they are therefore twice as wide as are the walls. Passageways (marked *m* on the plans) permit the ants to go from one room to another, as is necessary when a room is to be cleaned. The admission of strong light will insure the removal of the ant family to the darkened chamber adjoining. The passageways may be from one-fourth to one-half inch in width, and they should be covered with very thin glass, well glued on.

The tops of the walls and main partitions are exactly covered with coarse Turkish toweling, cut in strips twice as wide as the base on which it lies, and doubled so that its raw edges meet in the middle of the glass it doubly covers. The under layer of the toweling is glued to the glass, and it performs the double office of keeping the ants within the nest and of admitting sufficient fresh air for their breathing. Just over the passageways the toweling is left free, so that it may there be lifted for observation of what is within. After the toweling is laid on, the rooms have a uniform depth of less than half an inch, and a hand lens can be focused upon any of their inmates. The toweling being elastic and level, the roof-panes, of thin, clear glass, lie closely upon it. They are not fastened down.

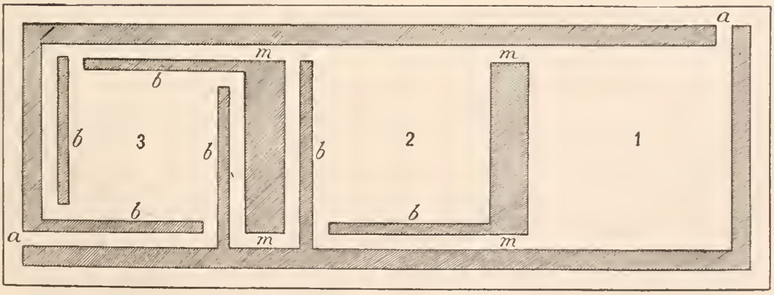
The roof-panes reach the center of the main partitions and



A. 6½ × 6 in. (16½ × 15½ *C.*)



B. 10 × 6 in. (25½ × 15½ *C.*)


a
m
m
b
b
3
b
b
2
1
b
b
a
m
m

C. 16½ × 6 in. (42 × 15½ *C.*)

1. Food-room. 2. Nursery. 3. Sponge-room.
a. Entrance. *b.* Screen. *m.* Passage.

project one-fourth of an inch beyond the walls. All the rooms except the food-room have an outer roofing of thick dark blotting paper, which should be lifted only when actual study of the ants is proceeding.

In the rooms numbered 1 the ants have, as in the Janet nests, a chance to range in the light and to seek food; of which it is well to put in the smallest sufficient quantity and several kinds. If the ants are made to move into the darkened food-room, leaving the other rooms free for cleaning, the passage-ways (*m*) may, during the cleaning, be plugged with cotton.

In room 3 a soft, fine sponge, clean and wet, and less than one-fourth of an inch thick, should nearly cover the floor, leaving a passage all around it next the walls. This furnishes drink to the ants and moisture to the air of their dwelling. If the ant young are in the egg or the larval stage, or if the temperature is high, the floor of room 2 should likewise be covered with wet sponge ; but if the young are in cocoons, or if the temperature is very low, then room 2 should have a layer of wadding instead of sponge. The ants generally choose damp places for the eggs and larvae, and dry places for the cocoons or pupae.

The screens (marked *b*) are substitutes for the ant-runs used in the ground, and they gratify the disposition of the ant to keep close to cover in going about in the nest. They are made in the same way as are the walls, but are only one-fourth of an inch thick, and are not topped with toweling.

The A nest, with base 6½ × 6 inches, is designed for a colony of very small ants, or for a few large ants. The B nest, with base 10 × 6 inches, affords a home for a somewhat larger family. The C nest, 16½ × 6 inches, can be used for a multiplying and dividing colony, or for observing the activities of restless species. The ants should never be greatly crowded in their habitation.

The ants in my nests appear sleek and healthy. I have found these nests easier than others to keep free from the molds that grow from particles of food that the ants convey from the food-room to every other part of their nest. These nests also lend themselves readily to experimental uses in studying the instincts of their occupants.

When the nests are to be carried on a journey the roofs are securely fastened down by sewing narrow strips of cheese cloth around the nest in such a way as to prevent the slipping of the roof-pane. The fastenings must not exclude fresh air. Having fastened the roof-panes each in place, the nests are put upon the appropriate shelves in the case, where they may be further secured by bits of wadding above the roof-panes and at the ends of the shelves.

The weight of my case, with its six enclosed nests packed for travel, is less than fifteen pounds. The strong local attachments of the ants are undisturbed by their so journeying, and at the end of the journey the study of their life processes may be speedily resumed.

Marine Biological Laboratory,
Woods Holl, September, 1900.

Volume II.] *December, 1900.* *[No. 3.*

BIOLOGICAL BULLETIN.

THE OESOPHAGEAL GLANDS OF URODELA.

R. R. BENSLEY.

FOR a long time the only known instance of glands occurring in the oesophagus of an Amphibian was the familiar pepsin-producing glands of the frog's oesophagus, discovered as early as 1838 by Bischoff (1). In 1853 Leydig (8) described the occurrence of saccular glands in the oesophagus of Proteus anguineus, and, more recently, similar glands have been discovered by Kingsbury (5) in the oesophagus of Necturus maculatus.

In no other Batrachian has investigation revealed the existence of glands in the oesophagus, unless, indeed, as Klein (6) suggests, the highly branched glands found at the junction of oesophagus and stomach in Triton, and termed by Langley (7) the anterior oxyntic glands, are such.

Naturally, considerable interest has been evinced in the question of the homology of these glands one with another, and with those of the higher vertebrate classes.

In order that the problem to be solved may be clearly understood, it may be as well to recapitulate briefly the facts as they appear in the forms so far investigated.

In the frog, leaving out of consideration the pyloric glands, there are two kinds of glands occurring in the foregut. The oesophageal glands occur under a ciliated epithelium, and are large compound glands, consisting each of a number of short tubular acimi lined by pepsin-secreting cells, opening into a common duct lined by transparent mucous cells. As we pass down the oesophagus we find that, at the point where the ciliated epithelium is succeeded by the ordinary mucigenous epithelium of the stomach, there is a gradual transition from the compound oesophageal glands to the more simple tubular glands of the stomach, the second type. The secreting cells of the two kinds of gland differ markedly from one another. Those of the gastric glands contain few zymogen granules of small size, while those of the oesophageal glands are more or less filled with very large granules, and the cells themselves are larger. Further, the oesophageal glands yield an alkaline secretion, the gastric glands an acid secretion.

In Triton, again, there are two types of gland. At the junction of oesophagus and stomach occur the anterior oxyntic glands of Langley. The difference between these glands and the other gastric glands (posterior oxyntic glands of Langley) is not so marked. The former are more highly branched and are separated from one another by a larger amount of connective tissue, but the differences in the size of the granules and in the nature of the secretion, so conspicuous in the case of the frog, are absent.

In Proteus a new structure makes its appearance in the shape of isolated sac-like glands occurring in the oesophagus. These have been fully investigated by Oppel (11), who describes them as follows : "Die Drüsen des Oesophagus haben eine rundliche Form. Sie bestehen aus einem grossen Acinus. Die Drüsen sind zusammengesetzt aus einem Ausführungsgang und dem secernierenden Theil. Ich spreche von einem Ausführungsgang, da sich die Zellen desselben von denen der Schleimhautoberfläche unterscheiden. Der Ausführungsgang besteht aus Zellen von annähernd cylindrischer Form, und zwar ist die Grenze zwischen conischem und cylindrischem Epithel stets eine scharfe. Eine besondere Eigenthümlichkeit liegt in der Uebergangsstelle von diesen cylindrischen Zellen des Ausführungsgangs zu den secernierenden Zellen. Dieselbe liegt nämlich nicht an der Stelle, an welcher die Erweiterung des engen Ganges zum Acinus stattfindet, sondern die Cylinderzellen gehen noch ein Stück weit in den Acinus hinein, um dann rasch zu den niedrigeren secernierenden Zellen abzufallen.

Diese Zellen zeigen in ihrem Protoplasma einen körnigen Bau, Körner, welche sich mit verschiedenen Farben, z. B. Eosin, S.-Fuchsin tingieren, mit Osmiumsäure bräunen . . . ." No glands resembling the anterior oxyntic glands of Triton are present in the adult, but he found in the young animal, at the junction of oesophagus and stomach, glands which are intermediate in nature between the oesophageal and gastric glands.

In Necturus there are, according to Kingsbury, three kinds of glands present. In the oesophagus are large saccular glands in most respects like those of Proteus, except that Kingsbury was unable, even after repeated trials, to demonstrate the presence of any granules capable of reducing osmic acid. At the junction of oesophagus and stomach are richly branched glands like the anterior oxyntic glands of Triton, and finally there are the ordinary gastric glands.

There are thus three types of gland occurring in the oesophagus of Batrachia, the relationship of which to one another, to the gastric glands, and to the oesophageal glands of higher vertebrates, is obscure. These are the compound pepsin-forming glands of the frog's oesophagus, the saccular glands of the oesophagus of Proteus and Necturus, and the anterior oxyntic glands of Triton and Necturus. It might be claimed for *a priori* reasons that no possible relationship could exist between the oesophageal glands and the gastric glands, but that position would necessitate a critical examination of the data on which this anatomical division of the foregut in the forms mentioned has been decided.

The writer found that Amblystoma combined, in a sense, the conditions found in Proteus and Triton, inasmuch as the glands in the larva resemble those of Proteus, the glands of the adult those of Triton. The present memoir is a brief account of the histogenesis of the glands in question.

Before passing on to a consideration of the histogenetic phenomena it is necessary to describe briefly the structure of the mucous membrane of the foregut in the adult animal.

The oesophagus is non-glandular, and is lined throughout by a ciliated epithelium, in which many goblet cells may be recognized. The ciliated epithelium is succeeded by the ordinary cylindrical cells of the stomach at the point where the first glands appear, and the oesophagus expands suddenly into the stomach. The ciliated epithelium does not extend into the stomach.

The gastric zymogenic glands are of two kinds. The anterior oxyntic glands occupy the proximal portion of the mucous membrane and form a zone about 2 mm. in width around the oesophageal orifice of the stomach. They are much shorter than the other gastric glands and, like the corresponding glands in Triton, are more highly branched, and contain more mucous neck cells. The rest of the stomach, with the exception of the posterior third in which the pyloric glands are found, is occupied by the usual tubular glands, consisting of a body composed of granular pepsin-forming cells, a neck composed of transparent mucous cells, and a duct composed of cells resembling the surface epithelium. These glands correspond in all respects to the excellent description given of the corresponding structures in Triton by Carlier (2). To make clear the terminology employed, a bi-tubular gland is represented in Fig. 1.

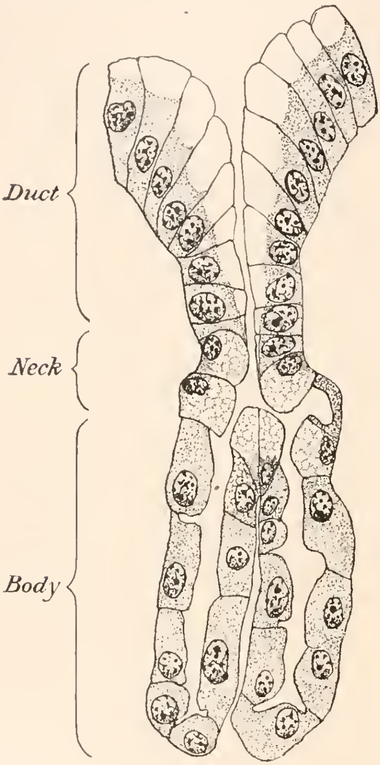


Fig. 1. — Gastric gland of Amblystoma Jeffersonianum. Zeiss apoch. 2 mm., ocular 2.

It was found necessary to resort to a new method of staining the zymogen granules, as the conventional method, by the employment of osmic acid, was not satisfactory when the cells contained brown pigment, or a great deal of prozymogen, which, as Langley (7) and Grützner (3) point out, also reduces the osmic acid, obscuring the granules if they be few in number or of small size. For this purpose the writer employed Reinke's neutral gentian as follows: To a saturated solution of gentian violet in water a solution of orange G is added in excess. A brownish precipitate is formed which is very slightly soluble in water. This may be collected on a filter and washed until the wash water is only slightly tinged. The precipitate is then dissolved in alcohol. For use a sufficient quantity is added to twenty per cent alcohol to make a fluid of about the same color as a good solution of haemalum. Sections fastened to the slide are stained in this for twenty-four hours, all adherent stain is then removed by pressing down upon the sections several folds of filter paper, absolute alcohol added and quickly removed with the blotter, and finally oil of cloves added in which the differentiation of the stain takes place. As soon as the protoplasm of the epithelial cells appears orange, the extraction of the stain may be checked by washing in benzole, and the sections may then be mounted in the usual way in balsam. The zymogen granules are stained of an intense blue color, the nuclei blue, other portions of the cells orange. The stain is most successful after fixation in aqueous sublimate.

The earliest stages in the formation of the gastric glands are difficult to discern, owing to the great number of yolk spherules present which obscure the outlines of the cells. In a larva 11 mm. long the glands are already visible as tubular down-growths of the endoderm of the foregut. In this early larva two kinds of glands are already to be recognized, those occupying the anterior end just behind the tracheal groove, and those at the posterior end, where the stomach is as yet not clearly marked off from the general endoderm. The anterior glands are of a flask-like shape, and have a distinct lumen surrounded by a single layer of cells. Zymogen granules are not yet to be recognized, and the yolk spherules are so abundant that the outlines of the cells are not visible. In the lumen there may often be seen one or two cells, which have been, so to speak, squeezed out of the row of endoderm cells forming the gland. These cells do not take any part in the formation of the permanent histological elements, but may often be recognized, even to a late stage of development, as disintegrating remains in these and the other gastric glands.

The posterior glands are simple tubes composed of a single layer of large yolk-filled cells surrounding a cleft-like lumen. Indeed, often it appears as if the endoderm of the foregut were in several layers without any differentiation into glands and epithelium. On careful inspection, however, it may be seen that the nuclei are arranged in an orderly fashion, as if surrounding the lumina of glands. Such an appearance is illustrated in Fig. 2. In this gland, in addition to the nuclei which are clearly arranged in a row around the lumen, two others may be seen which are nearer the center of the gland ; these are the nuclei of cells which will later be found as disintegrating remains in the lumen.

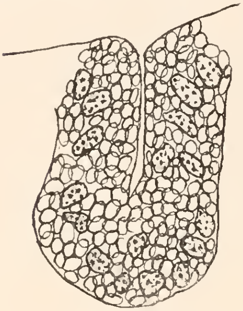


FIG. 2. — Amblystoma larva 11 mm. in length; gastric gland. Zeiss apoch. 2 mm., comp. ocular 2.

The flask-shaped glands do not, as one proceeds caudad, abruptly give place to the simple tubular glands, but there is a gradual transition.

In a larva 12 mm. in length, although the caudal portion of the stomach is still undifferentiated and the cells crowded with yolk, the yolk has sufficiently disappeared from the anterior portion to enable the shape of the glands and the cells composing them to be clearly determined. The anterior glands are now distinctly saccular, with a large lumen surrounded by a single layer of cells. The yolk spherules disappear from the cells of the glands somewhat more rapidly than from the surface epithelium, which as yet contains a considerable number. Notwithstanding the presence of the yolk, one can clearly distinguish, at this early stage, several kinds of cells, which can be readily referred to their analogues in the glands of the adult. The flask-shaped body of the gland (Fig. 3, *a*) is formed of a single layer of small cells, which vary from cubical to fusiform in shape and are usually convex towards the lumen. The protoplasm of these cells is granular and contains one or more yolk spherules. The nucleus is round or oval and rich in chromatin. As the gland narrows into the duct (Fig. 3, *b*), these are replaced by two or three slightly larger cells, in each of which two zones may be recognized, an outer, wider, deeply staining zone containing the oval nucleus, and an inner one which stains but feebly. This inner zone exhibits a reticular structure due to the presence of a secreted substance, probably mucigen. In the more posterior tubular glands, likewise, two kinds of cells may be found, similar in all respects to those of the flask-shaped glands, granular cells occupying the body of the gland, mucigenous cells the neck. In sections stained in Reinke's neutral gentian it is found that already numerous zymogen granules are present in the deeply staining cells forming the body of the gland. In the pancreas, also, zymogen granules may be recognized long before the yolk granules have entirely disappeared from the cells.

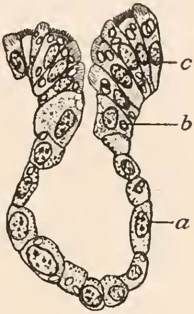


Fig. 3. — Amblystoma larva 12 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

The epithelium of the foregut in the region occupied by the flask-shaped glands is composed of two kinds of cells (Fig. 3, *c*), ciliated cells and cells the outer ends of which stain diffusely and intensely. These obviously represent the two characteristic elements of the future oesophageal epithelium, the ciliated and the goblet cells. Over the tubular glands farther back there is only one kind of cell in the epithelium, and this is without cilia.

Mitoses may be observed with equal frequency in all the various kinds of cell composing the epithelium and glands, and all are apparently equally capable of reproduction.

The important points to be learned from this stage are that the characteristic elements of the glands are differentiated very early, that no special groups of cells have, as yet, assumed the mitotic function to the exclusion of the others, and. that a portion of the glandular foregut bears a ciliated epithelium.

In a larva 14 mm. in length the foregut has advanced to a considerable degree beyond the stage last described. It is now shaped like a letter U, with a long proximal and short distal limb, the latter curving cephalad above the ventral posterior margin of the liver, before passing into the midgut.

Four regions may now be distinguished ; a very short anterior region without glands, provided with a ciliated epithelium, a region with flask-shaped glands and ciliated epithelium, a third region with tubular or saccular glands and a mucigenous epithelium, and finally, at the posterior end, a region in which no glands at all are to be discerned. The second and third regions gradually merge into one another, but the posterior non-glandular portion is sharply marked off and forms, in part at least, the future pyloric gland region.

At this stage the two pulmonary diverticula open into a capacious pouch lying below the foregut, into the floor of which it opens. In longitudinal sections the first gland appears immediately behind this sac. Farther back more glands make their appearance, and at the point where the foregut begins to enlarge into the stomach, it is completely encircled by six or eight of these large flask-shaped glands. Farther back again the glands become less and less flask-shaped and take on a tubular or saccular character.

One of these anterior glands is represented in Fig. 4 as seen after staining in haemalum, followed by neutral gentian. The shape of the cells in the body of the gland varies with the degree of distention. There seems to be in these glands an accumulation of the secretion in the lumen distending it, for it is only by the application of a distending force from within that the extreme stretching of the cells, which may be commonly observed, could be produced. In many glands where this distention is great the cells are quite flattened and spread out over a great surface, reminding one strongly of the appearance in the mammalian blastodermic vesicle at the time of its rapid expansion. The explanation there of the flattening of the cells is clearly the stretching caused by the rapid transudation of fluid into

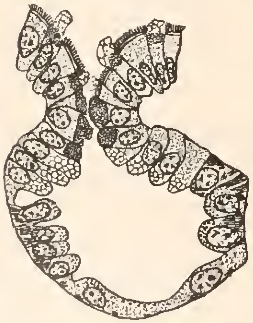


FIG. 4.—Amblystoma larva 14 mm. in length; oesophageal gland. Apoch. 2 mm., comp. ocular 2.

the vesicle, but in these glands it is difficult to explain why the fluid is not discharged into the cavity of the foregut before the pressure gets sufficiently high to cause a stretching of the cells. A possible explanation is the viscidity of the secretion owing to the large number of mucous cells in these glands.

Fig. 4 shows a gland only moderately distended, and here it is seen that the cells at the bottom, where the gland is unsupported by neighboring glands, are drawn out flat, while those at the side still retain their approximately columnar shape.

The two kinds of cells noticed in the earlier larva may still be recognized, the clear mucous cells occupying the top and neck of the flask, the granular cells the sides and base of the flask. The protoplasm of the latter now stains strongly in haematoxylin, and exhibits a faintly striated or finely vacuolated structure. This is due to the presence of prozymogen, which may also be demonstrated by the use of acid alcohol, followed by aqueous haematoxylin after the method of Macallum.[1] The inner end of the cell between the nucleus and the lumen stains but slightly in haemalum, but in sections treated with neutral gentian it is seen to be filled with perfectly round, deeply stained granules of zymogen. The neck of the flask-shaped gland is occupied by long mucous cells of a columnar shape, which also extend into the gland and form the top of the flask. In these cells two zones may be distinguished, an outer protoplasmic, which stains strongly and which contains a quantity of masked iron, and an inner transparent and reticular. The meshes of the latter are filled with a substance which stains faintly in indulin, more readily in Mayer's mucicarmine. In sections stained with neutral gentian many deeply stained granules may be seen in the mucigenous portions of these cells. These are somewhat elongated and not perfectly round, as are the zymogen granules of the other kind of cell. Their significance is not clear ; it is possible that they may indicate an imperfect differentiation of the zymogenic and mucigenic functions at this stage of development.

The surface epithelium in this region of the foregut consists of alternate ciliated cells and goblet cells. Tracing the foregut backward, the glands become gradually tubular or saccular, without any appearance of distention, and the ciliated cells disappear, so that the rest of the glandular portion, as well as all the posterior non-glandular portion of the stomach, is provided with a mucigenous epithelium.

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[1] *Journ. of Phys.* Vol. xxii. 1897.

Attention should be called at this stage to the remarkable resemblance between the mucigenous border of the gastric epithelium and the cuticula of the cells in the buccal cavity. Both have a characteristic striated appearance, and one is tempted to think that they cannot be very different chemically.

The cells of the tubular glands do not differ in any respect from those of the flask-shaped glands. The mucous cells are less numerous, and a few glands may be entirely without them. The cells of all the glands, even the very last, contain both zymogen granules and prozymogen.

There are as yet no pyloric glands formed; the epithelium of the posterior portion of the stomach is perfectly smooth and without glandular outgrowths.

Even at this stage there is a remarkable resemblance between the anterior flask-shaped glands and the oesophageal glands of Proteus and Necturus, and as development proceeds this resemblance becomes more and more striking.

The mouth and pharynx are lined in the aquatic Amblystoma larva by a stratified non-ciliated epithelium, with cuticular cells and goblet cells. In a transverse series it may be seen that immediately behind the last gill slit this changes to a ciliated epithelium. One may thus consider the first ciliated cell in a longitudinal section as indicating where the oesophagus begins. Measured from this point the foregut in a larva 16 mm. long is about 3 mm. in length. Of this .49 mm. at the anterior end is non-glandular. Behind this we have a portion .45 mm. long extending from the anterior border of the first gland to a point where the foregut begins to expand to form the stomach. This would doubtless, but for the presence of glands, be regarded as a portion of the oesophagus. Beyond this again, the ciliated epithelium extends into the stomach for a distance of .35 mm. The rest of the stomach is lined by a mucigenous epithelium, like that of the adult except in its great capacity for division. The posterior portion 1.3 mm. in length is still quite devoid of glands.

Staining with haemalum and neutral gentian shows that at this stage also the cells forming the body of every gland in the foregut contain both abundant zymogen granules and prozymogen, and it is impossible to discern any difference whatever in the cells composing the large anterior flask-shaped glands and the smaller posterior tubular glands respectively, except that in the latter there is no evidence of distention and consequent flattening.

From this stage onward the changes proceed somewhat more slowly and may be summed up briefly.

In a larva 25 mm. in length the foregut measured 5.6 mm. in length. Of this the anterior 1.4 mm. was non-glandular, showing a relatively more rapid growth in length in this portion of the foregut. The ciliated epithelium extended a further distance of .84 mm. into the stomach, the posterior portion of which, 3.36 mm. in length, was lined by the usual mucigenous epithelium.

Fig. 5 is from a larva 32 mm. in length. It is at this stage that the resemblance to the oesophageal glands of Proteus and Necturus is most marked. The duct of the gland and the portion of the wall nearest to the surface epithelium are composed of elongated cylindrical cells forming a single row. Four of these cells are represented in Fig. 6, *A*, as seen under a high magnification. Each presents an outer granular protoplasmic zone in which the oval nucleus is imbedded, and an inner more extensive zone which is coarsely reticular. They obviously represent the large, clear mucous cells of the ordinary gastric glands, and, as we shall see, are actually transformed into these in the adult. The difference in shape is dependent on external conditions, such as the grouping of the cells, and is not inherent in the cells themselves.

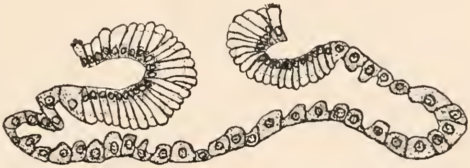

Fig. 5. — Larva of Amblystoma 32 mm. in length; oesophageal gland. Ross obj., $\frac{4}{10}$ in., Leitz ocular No. 1.

At the end of the gland they are succeeded suddenly by the zymogenic cells. In these the minute structure is obscured by the large amount of prozymogen present. The cells are less flattened than at the earlier stage of development, probably because the initial distention has been compensated by the rapid division and growth of the cells. A number of these cells is represented in Fig. 6, *B*. They are now somewhat columnar in shape, with convex ends projecting into the lumen. The nucleus is round or oval and placed in the center of the cell, though in the more columnar cell it is often nearer the lumen than the base of the cell. The free end of the cell may be seen in sections stained with neutral gentian, to be filled with granules of zymogen. Other granules may be seen at the sides of the nucleus, and a few are occasionally found in the base of the cell. The rest of the cell is occupied by a deeply staining protoplasm, which owes its ability to absorb nuclear stains to the large amount of prozymogen present, as may be shown by the employment of Macallum's methods of detecting masked iron. The distribution of the prozymogen determines the appearance of the cell, and three main types are to be recognized; in the first the stain is diffused through the whole of the protoplasm, but more pronounced at the base and sides of the cell, and on close examination a very finely vacuolated structure may be made out; in the second the whole or part of the cell exhibits long deeply staining fibrillae; and in the third type the prozymogen is distributed as small irregularly staining particles throughout the protoplasm.

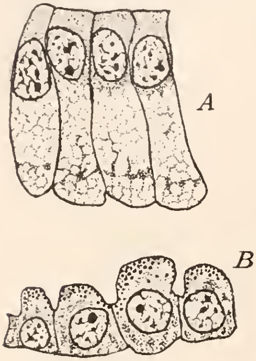

FIG. 6.—Oesophageal gland of Amblystoma larva. *A*, mucous cells; *B*, zymogenic cells. Zeiss apoch. 2 mm., comp. ocular 8.

All the three main types of cells composing the glands and surface epithelium are still capable of division, and numerous mitoses may be seen in all.

Oppel's description of the structure of the oesophageal glands of Proteus would apply word for word to these glands of the larval Amblystoma, and in the case of Necturus I have satisfied myself, by comparison of the actual objects, that the structures are identical.

The latest larva examined was 65 mm. in length. This animal was apparently about to undergo metamorphosis, as the stratified epithelium of the mouth had been replaced by ciliated. The pyloric glands were fully developed, and the ordinary gastric glands had assumed the appearance they present in the adult. The anterior portion of the stomach, about a millimeter in extent, was still ciliated, but the saccular glands of this region had undergone considerable modification. One of these is shown in Fig. 7. It will be seen that the base of the gland has grown out into a number of short secondary tubules, formed for the most part of zymogenic cells, and the gland now consists of a large number of such tubules, each similar in structure to an ordinary gastric gland, opening into a large common cavity lined by transparent mucous cells corresponding to the neck cells of an ordinary gland. In short, the saccular gland of the embryo is being transformed into an anterior oxyntic gland of the adult.

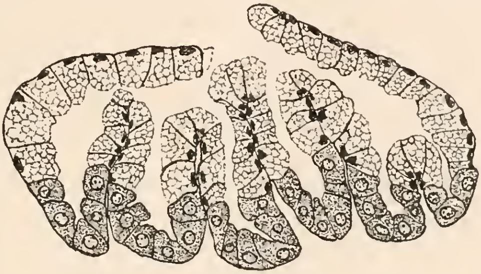

Fig. 7. — Oesophageal gland of 65 mm. larva. Ross obj. $\frac{1}{10}$ in., Leitz ocular No. 1.

Two of the most anterior glands in this larva were included in the oesophagus. All were in full physiological activity and were filled with zymogen granules.

I have been unable to secure a specimen of Amblystoma undergoing metamorphosis, or one that has just completed it, and am therefore unable to state positively whether all the saccular glands become transformed by subsequent branching into anterior oxyntic glands, or some of them degenerate and disappear. There is in the latest larva that I have examined no evidence of changes of a degenerate nature, and I am therefore inclined to believe that the most anterior glands, as well as the rest, are taken up into the stomach, and that the oesophagus of the adult is entirely formed by rapid growth from the short non-glandular region of the early larva.

One cannot doubt that the large saccular glands of the larval Amblystoma are the homologues of the so-called oesophageal glands of Proteus and Necturus. The failure of Kingsbury, however, to detect the presence of zymogen granules in the glands of Necturus led me to reinvestigate these structures with a view of determining whether or not this was a real point of difference. At first I employed a number of specimens of Necturus which had been kept in the laboratory tank for several months without food. In these cases the results were negative, no zymogen granules were present. I afterwards obtained two specimens captured in the vicinity of Toronto, and in a perfect state of nutrition. In these no difficulty was experienced in demonstrating the presence of zymogen granules in the cells of the oesophageal glands. Kingsbury's failure is, in all probability, to be ascribed to the inadequate method he employed to demonstrate the granules. For this purpose he employed treatment of the fresh glands with osmic acid. Now it has been noted by Langley (7) and Grützner (3) that the protoplasm of ferment-secreting cells which contain a great deal of reserve material (prozymogen) stains strongly in osmic acid. For this reason its use, in cases where the granules are few in number and small, and where there is a great deal of prozymogen present, is of little value. This is precisely the condition in Necturus. By the neutral gentian method, however, the granules are stained much more strongly than the prozymogen, and no difficulty is experienced in demonstrating them when present. Fig. 8 shows a number of cells from such a preparation.

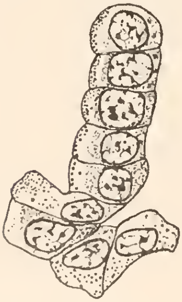
FIG. 8.—Zymogenic cells from oesophageal gland of Necturus, showing zymogen granules in free border of cells. Zeiss apoch. 2 mm., ocular 8.

It should be added, however, that the oesophageal glands of Necturus do not present the evidences of strong functional activity seen in those of the larval Amblystoma. The granules are much smaller and may be quite absent from many of the cells of the gland, even in a well-nourished animal. This is particularly the case in those cells the inner ends of which exhibit signs of degeneration in the shape of the structures described by Kingsbury as mucous globules. It is probable that there is a tendency for these glands in Necturus to degenerate rather than remain of physiological importance.

The so-called oesophageal glands of Proteus and Necturus are really gastric glands the development of which has been arrested. There is also in these animals an arrested development of the foregut, compensation for which has been, in a measure, attained by the conversion of the anterior portion of the stomach into a functional oesophagus. Only a short anterior non-glandular portion actually corresponds to the oesophagus of other Urodela.

Two questions remain to be considered, the relation of these glands to the oesophageal glands of higher vertebrates and to the oesophageal glands of the frog.

The first question is a comparatively simple one. The oesophageal glands of higher vertebrates have no features in common with those of Batrachia and are probably of secondary origin. In Reptilia oesophageal glands are rare, and where they do occur, as, for example, in Testudo graeca, are simple crypts lined by cells similar to those of the surface epithelium, namely, ciliated cells and goblet cells, the latter predominating. In birds and mammals, where the epithelium is usually of the stratified squamous variety, they are more or less complex mucous glands. In no case, as far as I am aware, has investigation revealed in the oesophageal glands of Sauropsida or Mammalia the occurrence of ferment-secreting cells. It is probable that the oesophageal glands of higher vertebrates have arisen in response to a demand, in a very long and relatively narrow oesophageal tube, for a more efficient lubricating mechanism, and an epithelium that will withstand friction. The first step in this process is the formation of deep crypts lined by ciliated cells and many goblet cells; the second, the disappearance of the ciliated cells from the crypts so as to form a pure mucous gland, and their replacement on the surface by a stratified squamous epithelium.

The second problem is less simple. Because of the very exceptional conditions introduced in the case of the frog by the herbivorous diet of the tadpole, and of the very extensive histolytic changes which take place in the whole intestine during metamorphosis, it becomes difficult to discuss this question from the standpoint of histogenesis. The question is, whether the oesophageal glands of the frog, like those of Proteus and Necturus, are to be regarded as somewhat modified anterior gastric glands. Let us examine, in the first place, the anatomical characters on which the subdivision of the foregut has been determined in this form. According to Wiedersheim (12) the stomach begins at a point where the foregut experiences an abrupt turn to the left. This is found on examination to correspond to the point where the ciliated epithelium is succeeded by the cylindrical epithelium of the stomach. There is also a slight constriction at this point and a change in the color of the mucous membrane. Of these the only character of importance is the change of epithelium. This is not, in my opinion, a valid criterion for the following reasons: In Amblystoma ciliated epithelium is found in the anterior portion of the stomach up to a late stage of development. In the tadpole, according to Gage, the whole foregut is ciliated, and several observers record patches of ciliated cells in the stomach of the adult frog. In several of our American "ganoids," Hopkins (4) and Macallum (9) describe the ciliated epithelium as being continued over a considerable portion of the stomach.

It is true that the differences in the cells of the oesophageal and gastric glands of the frog are very striking; but if we compare the oesophageal glands of the frog with the gastric glands of any Urodele or of Bufo, these differences are not apparent. The same cellular elements are present, with almost the same arrangement and structure.

The gastric glands of the frog are, in fact, unique among the Batrachia, in the small amount of zymogen which they contain. May this not be but another instance in which this animal, as compared with other Batrachia, exhibits an unusual degree of specialization, the anterior gastric glands (so-called oesophageal glands) having retained and developed the zymogenic function at the expense of the oxyntic function, and the posterior the oxyntic at the expense of the zymogenic function, thus foreshadowing in a parallel way the histological differentiation which is seen in the chief and parietal cells of the gastric glands of mammals?

The conditions obtaining in the foregut of Proteus, Necturus, and the larval Amblystoma are of interest apart from their purely histological bearing. For it is obvious that, if the condition in these animals is primitive, the gastric glands of the ancestral types must have occupied a much more extensive portion of the foregut than is the case in existing forms.

Among fishes the subdivision of the foregut into oesophagus and stomach is well marked, not only among the more highly specialized Teleostomes, but also in the sharks and rays. No glands are present in the oesophagus, and the epithelium is different from that of the stomach. In Amia, Lepidosteus, and Acipenser, according to Macallum (9), it is not only extremely difficult to decide on superficial examination where the oesophagus ends and the stomach begins, but on microscopic examination the former is found to have a similar epithelium to the stomach and to contain glands. The nature of these glands is at present in doubt. No doubt the investigation of the structure and histogenesis of the elements of the foregut in these forms, and more particularly in Polypterus, will yield highly interesting and instructive results.

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AN EXPERIMENTAL DEMONSTRATION OF THE REGENERATION OF THE PHARYNX OF ALLOLOBOPHORA FROM ENDODERM.

JOHANNA KROEBER.

Several recent investigators have shown with more or less probability that the lining of the new pharynx which develops during the regeneration of the head in certain earthworms comes from the endoderm, while the pharynx of the embryo is lined by ectoderm.[1] It seemed that by means of experimental methods this relation might be definitely determined. In the following pages I shall describe some experiments on Allolobophora foetida that demonstrate, I think, that the lining of the new pharynx is in fact derived from the endoderm.

Hescheler showed, as the result of observations made principally on Allolobophora terrestris, that when the five anterior segments are cut off the pharynx is regenerated by a growing forward of the old digestive tract up to the third segment, and that the new buccal cavity occupying the first three segments is formed by an ectodermal invagination. The old pharynx was not completely removed in these operations, since in the normal worm its cavity frequently extends beyond the fifth segment and its thickened muscular dorsal wall always goes back into the sixth, so that Hescheler's results are open to the objection that in his experiments *a part, at least, of the old pharyngeal wall always remained behind* as a possible source for the regeneration of the new pharynx.

Rievel in experimenting on certain Lumbricidae (Allolobophora foetida, Allolobophora terrestris, Lumbricus rubellus) cut off anterior ends consisting of between one-third to two-thirds of the entire body. He arrives at the conclusion that the pharynx is regenerated from the walls of the digestive tract at the point where this was cut, and that no ectodermal invagination whatever occurs, the endodermal diverticulum joining the body wall to form the mouth at the very anterior end of the worm.[1]

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[1] See Hoffman, *Zeit. f. wiss. Zool.* Bd. lxvi. 1899.

Haase showed that in Tubifex, when four to six anterior segments have been removed, the pharynx grows forward out of the walls of the digestive tract and meets an ectodermal invagination of somewhat varying size. This ectodermal pouch, which forms the buccal cavity, is small in all cases, never extending quite as far back even as the region of the cerebral ganglion.

Von Wagner's observations on Lumbriculus show that the point of union of ectoderm and endoderm, originally at the anterior end of the animal, subsequently comes to lie more posteriorly, on account of the forward growth of the "Kopflappen" and accompanying turning in of the ectoderm.

The differences in the accounts cited above show clearly that it is almost impossible to determine with certainty, merely by observation, just how much of the regenerated pharynx ultimately arises from the ectoderm and how much from the endoderm. It is very easy to see where ectoderm and endoderm meet, but the point of fusion is lost soon afterwards, and since the regenerated head continues to increase in size, it is presumably possible that the point of union may come to lie at some distance from its original position. At the time when the pharynx opens to the exterior its walls are not sufficiently developed for one to be able to determine whether the muscles will grow around the endodermal part of the tube ; but if in some manner the fusion of the ectoderm with the endoderm could be delayed long enough for the pharyngeal muscles to form around the latter, then the origin of the pharynx might be determined. Hescheler affirms that it is possible to make out the exact limits of the ectoderm by using stains which bring out the cuticle covering this layer. I used the stain which Hescheler mentions as giving the best results, but found that, while my preparations showed in general an agreement with the figures of Hescheler in regard to the extent of the cuticle on the dorsal wall of the pharynx, they contained also some alternating patches of what seemed to be cuticle and of ciliated areas on the ventral wall and at points in the digestive tract even further back than the regenerated pharynx itself.

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[1] For criticism of Rievel's results, see papers by Morgan (*Roux's Archiv*, Bd. v, 1897) and Hescheler (*Jenaische Zeitschrift*, 1898).

For this reason I have attempted to get more certain results by the use of the following experimental methods. Worms, from which the seven anterior segments had been removed, so that no part whatever of the old pharynx was left behind, were allowed to regenerate for a period of between twelve and eighteen days. As a rule the fusion of the ectodermal invagination with the pharynx occurs about fifteen days after the removal of the anterior end of the worm, — although there is considerable individual variation in regard to this point, and also some difference due probably to the temperature, etc. At the end of this time the anterior tip of the new part of the worm was removed in one of two ways: either it was burned off by touching it with a hot needle, or it was cut off with fine scissors. The latter method, though more difficult to carry out successfully, proved to be the better one because the piece cut off could be preserved to show whether the pharynx had joined the ectoderm at the time of the second operation. The worms were once more allowed to regenerate and were finally killed between ten and fifteen days after the second operation. In all cases the worms survived both operations and showed a perfectly normal regeneration, — the only point of difference from worms that had undergone only the first operation being that the new pharynx had time to regenerate before the second ectodermal invagination had fused with its anterior end. The object of the experiment was to determine whether a normal pharynx would develop from the endoderm if the fusion of the ectoderm with it was prevented for a sufficient length of time to allow this development to take place.

It is difficult to determine on the living object whether or not the ectodermal invagination has met the endoderm, and since for my purposes it was best to wait as long as possible before the second operation, it happened in two or three cases, as sections of the small pieces removed showed, that ectoderm and endoderm had met. In the majority of instances, however, I was fortunate enough to remove the invaginating ectoderm just in time. In cases where this was done with a hot needle there is, of course, nothing to prove that the fusion had not taken place. There is ground for such a belief, however, in the fact that, of a number of worms whose small anterior ends were cut off at the same time after the first operation as when the burning was done, and which were kept under exactly the same conditions, there was not a single one in which the fusion had taken place.

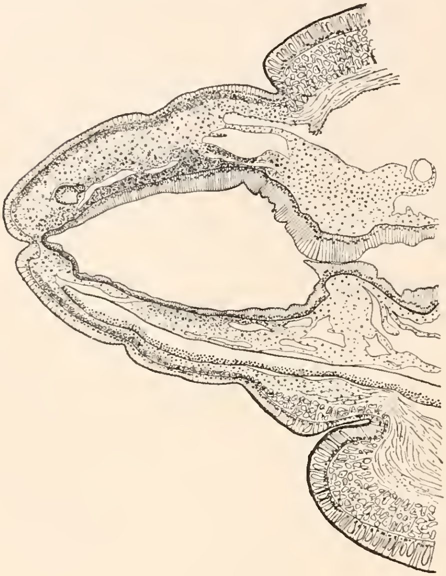


FIG. 1.

The same difficulty presents itself again in determining the time at which the worm is to be killed. I succeeded, however, in getting a number of cases where, though the pharynx and the ectoderm were just on the point of joining, they had not quite done so when the worm was killed. The accompanying figures show two worms in this condition.

Fig. 1 shows a vertical longitudinal section of a worm from which seven segments were removed on January 15. On February 2, that is to say eighteen days later, the tip of the newly regenerated part was cut off. This piece was preserved and sectioned and was found to include the whole of the ectodermal invagination besides the anterior end of the pharyngeal diverticulum which had not yet broken through to the exterior. Fourteen days after this operation, on February 16, the worm was killed.

Fig. 2 represents a vertical longitudinal section of a worm from which the first seven segments were cut off on January

15. The tip of the regenerated part was destroyed with a hot needle on February 2, and the worm was killed on February 17.

Both figures show the diverticulum which has grown out from the walls of the oesophagus about to open to the exterior by fusion with the ectodermal pit; and a comparison with the sections in the same neighborhood shows that these two represent the nearest approach of ectoderm and endoderm to be found in the two specimens. The walls of the pharynx and its musculature, especially on the dorsal side, are well developed. In both worms a nerve cord and a cerebral ganglion have been formed, the latter for the second time. Owing to the slight obliqueness of the section, as shown in Fig. 2, the nerve cord is cut for only a part of its length. The muscles of the body wall have begun to differentiate and there are clear indications of metamerism. All of the worms used in this set of experiments, as well as all those in a later set made to test these results, present a similar condition of things.

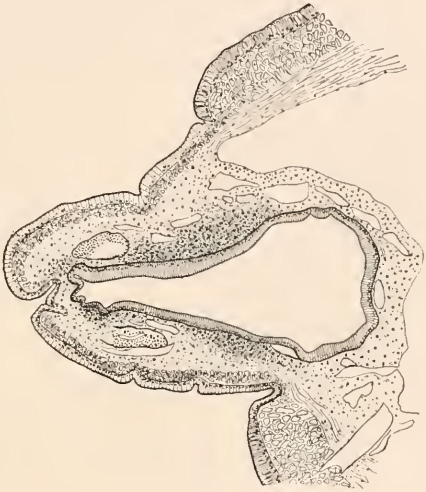


Fig. 2.

From these results we must conclude that the lining of the pharynx is regenerated from the endoderm, while the new ectoderm turns in for a very short distance to meet the pharynx and form the mouth.

The objection may be raised that the possibility of a later pushing in of the ectoderm to form the ultimate lining of the pharynx is in no way removed. But there is no evidence for such an occurrence and, even if it did take place, the fact remains that the musculature of the pharynx develops around an endodermal tube, as my experiments have shown, while in the embryo the lining of this same region is derived from the ectoderm.

The preceding work was done under the direction of Prof. T. H. Morgan, to whom I wish to express my indebtedness.

Bryn Mawr, May 26, 1900.

FURTHER EXPERIMENTS ON THE REGENERATION OF TISSUE COMPOSED OF PARTS OF TWO SPECIES.

T. H. MORGAN.

The experiments that I made a year ago were undertaken in order to find out if regenerated tissue, made up of cells derived from two species, showed any mixing of the specific characters of the two species. For this purpose I grafted the tail of a tadpole of one species of frog upon the posterior end of a tadpole of another species. Later the tail was cut off in such a way (as indicated by the line *b-b* in Fig. 5) that the ectoderm left at the exposed edge belonged in part to one species, in part to the other. When the new tail regenerated there was found to be no mixing of the characters of the ectodermal cells along their line of contact in the new part. The results were unsatisfactory from one point of view, inasmuch as the small piece of ectoderm left after the operation is carried out to the tip of the new tail and increases proportionally less in area than the rest of the new part, so that although it is highly probable that near the tip of the tail new ectodermal cells are being formed by both kinds of ectoderm, still I did not demonstrate that this is actually the case. Moreover, I found that in the later stages the difference in color between the two kinds of ectoderm was less marked than at first, so that the experiment would have been more convincing had the tail been cut off at an earlier stage. This I have done during the present spring, and the results in regard to the ectoderm

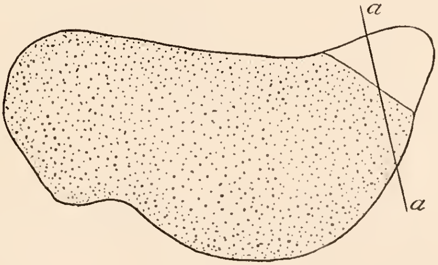


FIG. 1.

confirm in every way those given in my former paper. In the experiments made this spring my main object has been, however, to carry out the experiment in such a way that there would be left at the exposed edge, when the grafted tail was cut off, the internal tissues of two species. In this way I hoped to be able to determine more definitely if, in the newly regenerated part, the tissues mutually influence each other.

The day after the grafting had been performed (*i.e.*, after eighteen to twenty-four hours) the tail was cut off at the region of union of the two components, as shown by the line *a-a* in Fig. 1. In this way there is left at the exposed edge not only the ectoderm of the two species, but the inner tissues also. The regeneration that takes place from the exposed edge will include material derived from both components. Two possibilities presented themselves. First, would the new part be formed of cells intermediate in character between the two species as the result of an interaction of the cells on each other; or, second, would the new material preserve the characteristics of the region from which it arises, or, in other words, one-half of the tail show the characters of one species and the other half of the other species? It is further possible that the new cells might intermingle, and if so the tail might *appear* to be of a hybrid character.

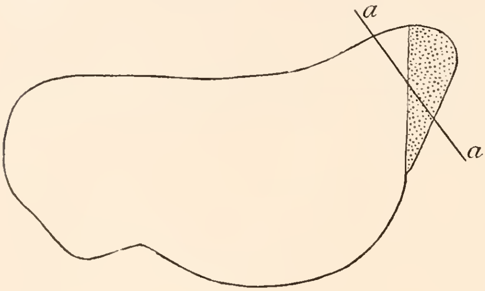
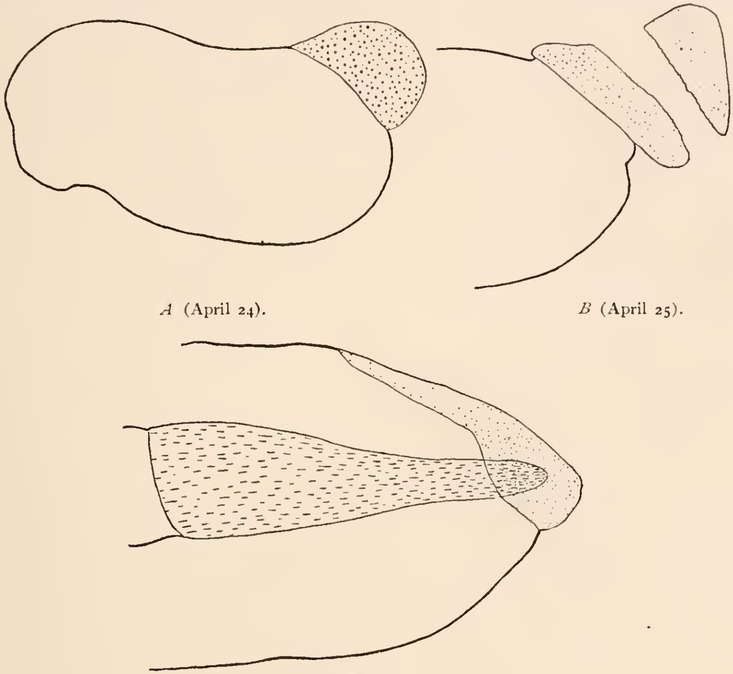


Fig. 2.

Other experiments of minor interest have also been studied. For instance, in several cases the grafted tail was cut off after twenty-four hours very near its line of union to the major component, as shown in Fig. 3, *A*, *B*. The experiment was made in order to see if the major component might not have some influence on the regenerated part from which it is separated by only a narrow band of tissue of the minor component, but no such influence was observed.

I have found a much safer criterion than before for distinguishing the inner tissues of the two species of tadpoles used in these experiments. In my former experiments I used the differences in color of the pigment cells. I find that this cannot be relied upon under all circumstances. But the muscle tissue of the tail of Rana palustris is, especially in the early stages, golden-yellow, while in Rana sylvatica the same cells are slaty-white. The two kinds of cells can be easily distinguished by means of this color difference. Other experiments have shown also that this difference in color is transmitted to the regenerating tissues in the new tail, so that it can be relied upon in the grafting experiments.


A (April 24). *B* (April 25).

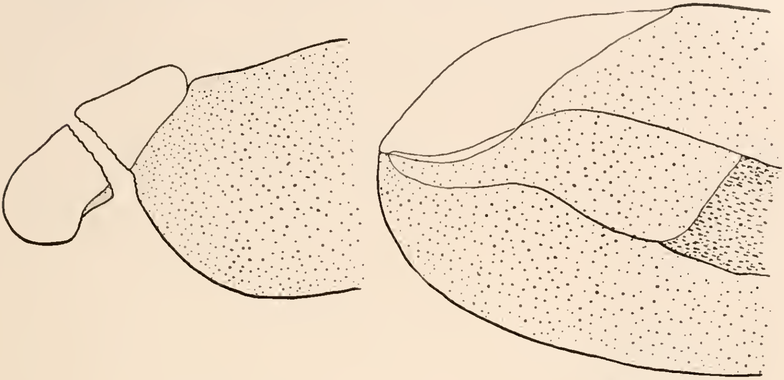
C (May 2).

Fig. 3.

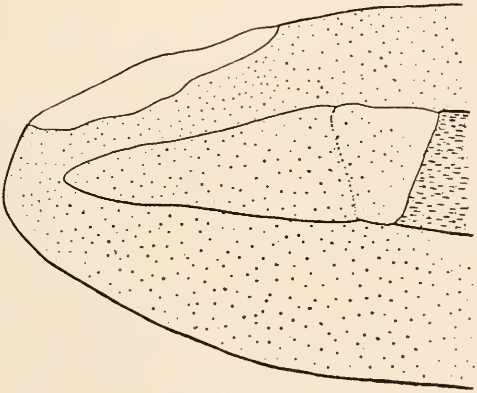
In the first series of experiments the tails were grafted as described in my former paper. After eighteen to twenty-four hours, as a rule, the tail was cut off, as shown by the line *a-a* in Fig. 1. Before grafting it was found more convenient to cut off the tails obliquely, as shown in the figure — the more anterior end on the dorsal side. Consequently, in order to carry out the second operation of cutting off the tail through the line of union, the cut was made also obliquely, but with the ventral side forward. In a few cases the tails were first cut off with the ventral side further forward (Fig. 2), and the subsequent cutting off was made with the dorsal side forward, as shown by *a-a* in Fig. 2, but the results were practically the same.

It was found easier to graft the tail of Rana palustris on the posterior end of Rana sylvatica than the reverse. On an average five operations of the former succeeded to one of the latter. The reason for this cannot be given, but it may be due to some difference in the relative sizes of the two components that is more favorable for union in one than in the other way. The result recalls the experiments in cross-fertilization of the eggs in different species, where the crossing can be more easily carried out in one direction than in the other. In this case also the results may be due in some cases to a gross, physical difference, as Pflüger has tried to show for the frog's egg.

In the large majority of cases in which the experiment was carried out as shown in Fig. 1, the core of the new tail seemed to be formed by the minor component, — *i.e.*, if a yellow tail (R. palustris) had been grafted upon a black tadpole (R. sylvatica) and then after twenty-four hours the tail had been cut off obliquely (Fig. 4, *A*), the central part of the new tail would be composed entirely of the yellow tissue derived from the minor component (Fig. 4, *B*, *C*). The small piece of yellow ectoderm is carried out on the new tail and remains near the tip. It covers a larger area than at first, but it increases not nearly so fast as the rest of the new, yellow tissue of the new tail. The distinctive differences in color can only be seen in the core of the tail, *i.e.*, in the cells that form the muscles. On each side of this axial core a broad fin is present containing inside a gelatinous-like substance with scattered cells, but this fin does not show any difference in color in the two species. It is, therefore, probable that in many cases in which the core of the new tail appears to be composed only of tissue from the minor component that the ventral (or dorsal) connective tissue of the fin is derived from the major component. The differences in the mesodermal pigment cells are at times very striking, and in all such cases the pigment cells are like those in the tissues from which they immediately arise; but while in many cases they furnish a safe criterion, in others the difference cannot be made out with certainty. On the other hand, the differences in the muscle tissue of the core can always be seen.



A (April 25). *B* (May 2).



C (May 6).

Fig. 4.

The explanation of the result, *viz.*, that the new tail is in most cases (in forty-seven cases out of sixty) like the minor component, is that in nearly all of these operations too large a piece of the grafted tail has been left. It has contained the notochord and nerve cord and the tissue immediately around them, and from these the new tail has grown out. I did not discover this until most of my material had been used. After this I cut off the grafted tails nearer the line of union, and although regeneration did not take place so well in several cases, still those that regenerated showed more often both parts contributing to the new tail. The same result followed in a number of cases in the previous operations, and we may now examine how regeneration takes place in such cases.

In thirteen cases (out of sixty) there was found evidence of a dual or compound character to the new tail. In all cases observed there was no evidence to show that the duality was the result of the tissues being mixed in character either by commingling of the cells (each cell retaining its specific characters) or by a hybridizing of the cells (due to mutual influence). The duality consisted in each part, regenerating cells like itself, so that definite regions of the new tail were made up of one or of the other kind of tissue. For instance, the new tail might be made up above of the slaty-colored tissue of R. sylvatica and below of the yellow tissue of R. palustris. There is no evidence of a shading of one kind of tissue into the other along the line of meeting, but this point would be very difficult to determine positively. There is further no evidence that the two kinds of tissue are any more commingled at the distal end of the tail than at the base.

In regard to the notochord and nerve cord it is extremely unlikely that the cut would ever pass obliquely through the line of union of the one or of the other, as these structures are very small in cross-section. It is, therefore, probable that in nearly every case the new notochord and the new nerve cord are made up of cells belonging entirely to one component. Furthermore, these two structures lie so near together that it is not probable that the cut would pass between them in such a way that the nerve cord at the exposed edge would belong to one component and the notochord to the other component.

The details of the successful experiments are as follows: On April 14 and 15 nine grafts were made, as shown in Figs. 1 and 2. On April 16 these were cut off, as indicated in Fig. 1, *a-a*, and Fig. 2, *a-a*, but unfortunately the two lots were not kept separately. On April 29 when again examined new tails had begun to regenerate, and two individuals out of the nine showed that the core of the new tail was compound in character. In both the major component was black and the minor yellow. In one of these the new tail was yellow on the dorsal side and black on the ventral, and in the other the new tail was black on the dorsal side and yellow on the ventral.

In another series the experiment was somewhat different. The grafting took place on April 17. Two days later the ectoderm of the minor component had been carried out further on the tail (Fig. 5), so that at the base of the tail the inner tissues of the minor component were covered by the ectoderm of the major component. At this time (April 19) the tail was cut off obliquely, as indicated by the line *a-a* in Fig. 4, leaving the inner tissues of both components exposed at the cut surface. On May 19 all three of the tadpoles that had been operated upon showed a compound tail. One of these tadpoles was R. palustris and the other two were R. sylvatica, with grafted tails of the other species, respectively.

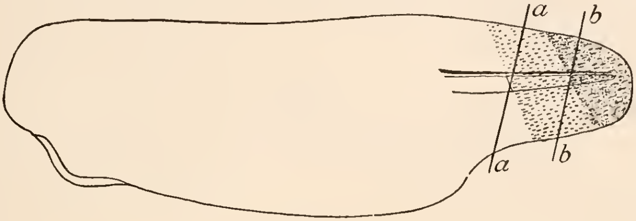


Fig. 5.

In a third series of five individuals, grafted April 19 and cut off April 20, as in Fig. 1, two showed later a compound tail; and in a third I was in doubt whether or not a few of the yellow cells of the major component entered the new tail.

In three later experiments in which the tail had been cut off, so that a smaller piece of the minor component was left attached, a larger number regenerated compound tails.

In one of these experiments the grafting took place on April 27, and the tail was cut off on the following day. One of the three produced a tail composed of both kinds of tissue.

In another experiment grafted April 28 (2.30 P.M.), as shown in Fig. 2, and cut off April 29 (10 A.M.), two individuals formed abnormal tails and a third a compound tail. The tail of this individual is represented in Fig. 6. On the ventral side of the new tail are found the slate-colored cells of the major component, and on the dorsal side the yellow cells of the grafted piece. (It is not possible to show this difference satisfactorily in a simple uncolored drawing, since the principal difference is one of color.) In addition to this difference one can see in the region at which the grafting took place and where the new tissue arises from the old that each component contributes its half to the new tail. Moreover, in all these cases the tadpoles had been carefully observed from day to day (and not only at the intervals recorded in the text) and the gradual formation of the compound tail observed.

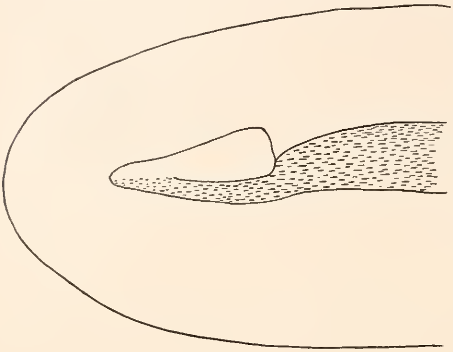


FIG. 6.

In another experiment on April 28 the tail was cut off on April 29. One of the tadpoles did not regenerate a new tail, another (R. sylvatica) had a compound tail, and one had a bifid tail, one branch being compound. Finally in another series in which nine grafts were made, one produced a compound tail, another may have contained a small amount of the major component in the new tail, six regenerated entirely like the minor component, and one was abnormal.

In addition to these cases there were three others (in the total of sixty cases) in which there was an overlapping of the two components in the tail, as in Fig. 6. In two of these the core of the new tail came from the minor component, but it is highly probable that a small addition came from the major component also. In the third case the new tail contained at its more distal end elements from both components. Unfortunately this lot was killed accidentally before they regenerated further.

In several cases double tails grew out enclosed in the same common fin, and lay usually in the same plane. In some cases the core of one of the new tails was derived from one of the components and the other from the other component. In several cases one or the other new tail received material from both components. In one of these cases it could be seen with the greatest clearness that the compound tail received material from both sources (Fig. 7). Cases of this kind are particularly convincing, since they furnish all the data for comparison between the two kinds of regenerating tissue of the two components. The dorsal tail was yellow and the upper part of the ventral tail was also yellow, and its tissue precisely like that of the dorsal tail. The pigment cells also of the yellow component extended out on to both tails.

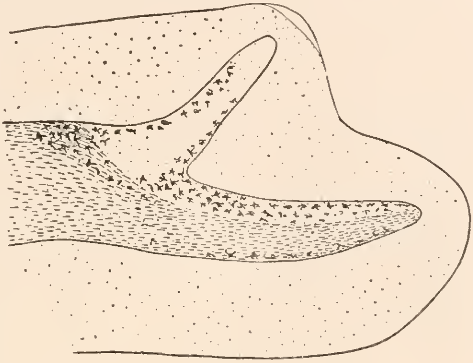


Fig. 7.

These cases of double tails are probably due to imperfect grafting, — the notochord and nerve cord of the two components not being in the same position, so that when the tail is cut off both sets of structures are exposed and a tail develops from each.

Conclusions. — The experiments demonstrate that a single tail may be formed by the regeneration of tissue derived from two species, and that in such cases there is no specific change produced in the one kind of new tissue by the other. Each kind of tissue regenerates its like, and the two kinds combine to form a single morphological organ, — the tail.

May, 1900.

REVIEW OF VON GUAITA'S EXPERIMENTS IN BREEDING MICE.

C. B. DAVENPORT.

In the two latest volumes of the *Berichte der Naturforschenden Gesellschaft zu Freiburg*, G. von Guaita gives the results of his breeding experiments. He started in 1896 with fifty-five *Japanese walzing* mice and with numerous *white* mice belonging to a race bred by Weismann since 1888, and made crossings through seven generations to 1900. His main data concern the inheritance of color; incidentally, data were got on the diminution of fertility with in-and-in breeding.

Diminution of Fertility.

These results were gained chiefly from Weismann's breedings. The total number of young, the number of litters, and the number of young per litter are given below for each decade of generations.

1st to 10th generation :	1345 young ;	219 litters ;	avg. young per litter, 6.1
11th to 20th “	352 “	62 “	“ “ “ “ 5.6
21st to 29th “	124 “	29 “	“ “ “ “ 4.2

In von Guaita's breedings:

1st and 2d generations, ...	“ “ “ “ 3.5
3rd and 4th “	“ “ “ “ 3.7
5th and 6th “	“ “ “ “ 2.9

Thus in the successive generations there is a reduction in fertility of about thirty per cent; and this is probably due to too close breeding.

Color of Mixtures.

Japanese walzing mice vary in color, but are chiefly piebald — black and white. White mice are without pigment (true albinos) and breed very true.

Crosses of walzing ♀ × white ♂ and white ♀ × walzing ♂ gave twenty-eight young. All were of a gray color and indistinguishable either in respect to color or size from the common house mouse. Also in temperament they were like the house mouse, for they were very wild and lively (unlike either parent) and the walzing action was entirely absent from all the mice of this second generation. Similar results were got by Haacke ('95) after crossing the same races. Haacke says: "When you pair a blue and white spotted walzing mouse with a common white mouse you get either (and usually) uniformly gray mice, which cannot be distinguished from the wild house mouse, or else (more rarely) uniformly black mice." These results, then, lead to the conclusion that when very unlike races of mice are crossed the result is often or usually a *reversion.*

A *third* generation was next produced by von Guaita by mating two of the gray mice or reversions. Four pairs were thus mated and forty-four young were reared — all having both parents gray, and half their grandparents walzing and half white. These fourty-four mice are placed in nine color classes, as follows:

				Per cent
"House" or *Gray* type.	entirely gray,	15	25	57
	gray with isolated markings,	7		
	black [essentially house type],	3		
Albino.	pure white, red eyes,		11	25
Walzing type.	white walzers,	3	8	18
	gray-white spotted walzers,	1		
	gray walzers,	2		
	black-white spotted walzers,	1		
	black walzers,	1		
			44	100

The most striking phenomenon of this third generation is the sudden occurrence of great variation. In the language of plant breeders "the *type* is broken." Plant breeders (*e.g.*, Focke and de Vries) have long ago observed that the progeny of hybrids is extraordinarily variable.

Fourth Generation. — Several pairs of the foregoing descendants of the reverted gray mice were mated, and thirty-one young sorted into eight classes were obtained, as follows:

Third Generation ☞	Gray ♀ White-Walzer ♂	Gray ♀ White ♂	White ♀ White ♂	Gray and Spotted ♀ Gray and Spotted ♂	Total	Sum	Per Cent
Uniformly gray . . .	2	12			14		
Gray with markings . .		2		2	4		
Black	2				2	20	65
Albinos	1		4			5	16
Uniformly gray walzers	1	1			2		
Gray walzers with spots		1			1		
Black-white walzers . .	1				1		
Black walzers	2				2	6	19
	9	16	4	2		31	100

As in the third generation, there is here great variation. The results may be generalized as follows:

(1) All descendants of albinic parents are albinos.

(2) When both parents are gray and spotted all descendants (2) are gray and spotted.

(3) Gray ♀ × white ♂ gives 88 per cent gray and 12 per cent walzers; the white is shut out.

(4) Gray ♀ × white walzer ♂ gives 44 per cent gray, 44 per cent walzers, and 12 per cent white.

Fifth Generation. — To save room we will henceforth make use of abbreviations for the names of colors, as follows:

G = gray.
Gw = gray with white markings.
B = uniformly black.
Bw = black with white markings.
A = albino or white.

Ww = white walzers.
Wg = gray walzers.
Wgw = gray-white walzers.
Wb = black walzers.
Wbw = black and white walzers.

The female parent invariably precedes the male.

Gen.	1	2	3	4	5	6	Total	Sum	Per Cent
III	(G♀ × Ww♂) × (G♀ × Ww♂)		(G×Ww) × (G×A)	(Gw×Gw) × (G×Ww)	(G×A) × (Gw×Gw)	4 Gw			
IV	Wb♀ × B♂	Bw♀ × B♂	Bw × Wg	Gw × Wb	Gw × Gw	Gw × Gw			
G			1				1		
Gw				2	35	16	53		
B		8		1			9		
Bw	2	10		7			19	82	73
A	1	7	1		9			18	16
Ww	1						1		
Wg			1	1			2		
Wgw				2			2		
Wb	1	2					3		
Wbw		5					5	13	11
	5	32	3	13	44	16		113	100

Sixth Generation.

Gen.	Colors					Total	Sum	Per Cent
II	All gray or reversions							
III	A, 2; Gw, 4; G, 2	A, 1; Gw, 2; G, 3; Ww, 2	Gw, 2; G, 3; Ww, 3		G, 4; Ww, 4			
IV	Gw, 4	Gw, 1; Bw, 1; Wg, 1; Wb, 1	Gw, 1; Bw, 1; B, 1; Wb, 1		Bw, 1; B, 2; W, 1			
V	Gw × Gw	Wgw × G	B × A	Wb × B	A × Ww			
G	1	1				2		
Gw	20	1				21		
B			7	1		8		
Bw			16	1		17	48	58
A	10		18		4		32	38
Ww			2			2		
Wb		1				1	3	4
	31	3	43	2	4		83	100

Seventh Generation.—The colors of only eight members of this generation were determined—too few to be significant.

General Results.

In the successive generations the percentage of walzing individuals undergoes a steady decline from eighteen per cent and nineteen per cent in the third and fourth generations to eleven per cent in the fifth and four per cent in the sixth generation. Is this decline due to the elimination of an unstable condition or to the circumstance that too little of the walzing blood has been employed in the later crosses to keep up the original proportion? The question whether the normal law of inheritance is followed here may, indeed, be asked of all the colors. The normal law of inheritance, as defined by Galton, is that one-half the heritage of any generation is derived from the parents, one-fourth from the grandparents, one-eighth from the great-grandparents, and so on, according to the formula:

$$\text{Inheritance} = \tfrac{1}{2}\,k_1 + \tfrac{1}{4}\,k_2 + \tfrac{1}{8}\,k_3 + \tfrac{1}{16}\,k_4 + \text{etc.}$$

To apply the normal law of inheritance it is convenient to assume it and to compare the theoretical heritage with the empirical. If the two agree, the validity of the law is established in this case; conversely, if the two do not agree, the law does not hold. This method of testing the law is the same as that employed by Galton ('98) in the case of Bassett hounds. It may be illustrated by the calculation of the theoretical number of albinos in the sixth generation. Let us take the first column. If one of the two parents were an albino, we should expect at least $\frac{1}{2} \times 50\%$ of the progeny to be such. If both parents were A, $\frac{2}{2} \times 50\%$ of progeny at least should be A. If, in addition, all of the grandparents (Gen. IV) were A, we should expect at least $\frac{2}{2} \times 50\% + \frac{4}{4} \times 25\%$ of the VI Gen. to be A. In general, if n_{v}, n_{iv}, n_{iii}, etc., represent the number of times an albino appears as ancestor in the different generations, then the proportion of albinos in the sixth generation should be:

$$\%A_{\text{vi}} = \frac{n_{\text{v}}}{2} \times 50\% + \frac{n_{\text{iv}}}{4} \times 25\% + \frac{n_{\text{iii}}}{8} \times 12.5\% + \frac{n_{\text{ii}}}{16} \times 6.25\% + \frac{n_{\text{i}}}{32} \times 3.125\% + \frac{1}{2} \times 3.125\%.$$

The last term is got by observing that the ancestors of half of the first generation were exclusively albinos for many generations, while the ancestors of the other half were exclusively walzers. The value of A is similarly calculated for each column, and the theoretical number of individuals for each column is found. Their sum should be equal to the observed number, or, when reduced to percentages, to the observed percentage of total. The closeness of theory to observation is sometimes striking. Thus if we compare column by column the observed and theoretical frequencies of walzers in the fifth generation we get:

COLUMN	1	2	3	4	5	6	TOTAL	PER CENT
Observed	2	7	1	3	0	0	13	11
Calculated	2.19	6.00	1.13	4.88	0	0	14.19	12.5

In the following table are given for each generation the observed and (in parenthesis) the corresponding calculated percentages for each color:

GENERATION	I	II	III	IV	V	VI
Black alone (B) . . .	0	0 (0)	7 (0)	7 (0)	25 (18)	30 (40)
Gray less black (G)	0	100 (0)	50 (50)	58 (48)	48 (60)	28 (28)
Total gray and black	0	100 (0)	57 (50)	65 (48)	73 (78)	58 (69)
Albinos	50	0 (50)	25 (25)	16 (32)	16 (9)	38 (18)
Walzers	50	0 (50)	18 (25)	19 (20)	11 (13)	4 (13)

Several remarkable things come out of this table. In the first place the most marked departure from Galton's Law of Ancestral Inheritance is seen in the second generation, where the gray, non-walzing reversions suddenly made their appearance. We know as yet little concerning the laws of the phenomenon called "reversion"; but whether it be considered a remote atavism or only an apparent "inheritance," it seems equally to form an exception to Galton's Law.

Secondly, the case of the walzers does indeed look like an exception to Galton's Law. It looks as though the walzing condition were an unstable condition being rapidly eliminated. In so far the result opposes the usual expectation of sport prepotency.

Thirdly, the albinos, likewise sports, apparently are prepotent, since there is twice the proportion there should be in the sixth generation. The numbers are so large that one can hardly object that these figures are not altogether significant.

Fourthly, the grays run close to theory, excepting always generation II. They are nearest to the original type of *Mus musculus* and seem to inherit in the most nearly normal fashion.

In conclusion, then, we may say that the data afforded by these breeding experiments indicate, so far as they go, that Galton's Law of Inheritance holds only with form units which are not very divergent from the type, and that among sports we may have some that show a great stability and prepotency, while we may occasionally have others which are physiologically so unfit that they are unstable and have less than normal potency.

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VARIATION IN THE TEETH OF NEREIS.

MARY HEFFERAN.

THE purpose of this quantitative study in variation is to determine the modal condition of a character in a species of Nereis commonly found on the west coast of the Atlantic. The material was very generously placed at my disposal by Professor Charles B. Davenport, who collected it during the summer of 1899 at Cold Spring Harbor, Long Island. The animals were found in the sand of the beach and were taken at random, excepting that small ones were rejected.

These annelids went by the familiar name of *Nereis virens*, but upon comparing them with Ehlers's ('68, p. 559) descriptions and drawings of that species, I found that although they agreed in most characters, an important difference occurred in the length of the first or postoccipital segment; that of *N. virens* being twice as long as the second segment, while that of the Cold Spring Harbor form is about equal to or even slightly less than the second in length. In this character, as also in that of certain parapodal bristles, the "Sichelanhänge," which are rather short and broad instead of slender and long as in *N. virens*, the Cold Spring Harbor species agreed well with Ehlers's description of *N. limbata*, the distribution of which also includes the west Atlantic coast. From these two points, and from the fact that Cold Spring Harbor is slightly south of the southern limit described for *N. virens*, and within the range of *N. limbata*, it seems probable that we are here dealing with the latter of Ehlers's two species. It may be possible that the two species overlap in this region at the southern limit of *N. virens*, and that my collection contained both. However, nothing in the numerical results of my investigation seemed to suggest two distinct or even transitional forms.

1. *Method.*

The specific character selected for investigation was the number of teeth on the jaw. This number is commonly stated by authors in descriptions of species.

The jaws are two in number, from 1 to 3 mm. in length, brown, horny, curved, and serrated along the inner or falcate margin. They are at the extremity of a large exsertile proboscis which is usually retracted in alcoholic specimens, so that in order to free the jaws it is necessary to cut down the median line of the head, ventrally, through to the inside of the muscular proboscis. By turning the head backwards the jaws can be made to extrude, and the teeth counted by means of a hand lens. In the specimens killed later in the season by a slowly killing fluid, the jaws remained extruded.

In counting, some difficulty was experienced in fixing a limit in either direction, at the curved, distal end in those cases in which very fine teeth ran to the tip, and particularly at the proximal extremity where the outlines of the teeth were indefinite. In order to count these it was necessary to pull out the jaws gently with a forceps, and to free the bases from connective tissue, carefully, without entirely separating the jaws from the head. Since the line of division between the free, prominent teeth and the undeveloped ones, buried in a chitinous band, was always distinct, the method was adopted of counting them separately, and of including in each set all that showed well-formed outlines when held up against a strong light and viewed through a lens from the dorsal side. Those connected by the chitinous band were called indefinite teeth, the rest the definite teeth. Totals were found by adding.

2. *Results.*

I. TABLE OF DISTRIBUTION OF FREQUENCIES.

Classes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
L. Def. . .		5	30	88	128	102	41	6						
L. Indef. .		12	30	93	146	93	21	4	1					
L. Total .						3	12	28	95	116	100	39	7	
R. Def. . .	1	4	37	94	126	86	41	11						
R. Indef. .	2	7	30	80	149	97	28	7						
R. Total .					1	2	8	27	102	114	89	46	10	1

From the table of frequencies it will be seen that the number of definite teeth varies from 1 to 8, the indefinite from 1 to 9, the total numbers from 5 to 14.

The following constants were obtained.

II. TABLE OF CONSTANTS.

	LEFT DEF.	LEFT INDEF.	LEFT TOTAL.	RIGHT DEF.	RIGHT INDEF.	RIGHT TOTAL.
n	400	400	400	400	400	400
M	5	5	10	5	5	10
A	5.098 ± 0.040	4.905 ± 0.039	10.00 ± 0.044	5.043 ± 0.043	5.013 ± 0.040	10.055 ± 0.045
σ	1.193 ± 0.028	1.179 ± 0.0279	1.306 ± 0.031	1.267 ± 0.030	1.191 ± 0.028	1.339 ± 0.032
F	+ 0.7025	− 0.66148	+ 0.0599	+ 0.4339	− 0.8617	− 0.5314
Type	I	IV	I	I	IV	IV
Skewness	− 0.0369	− 0.0439	− 0.1341	+ 0.0153	− 0.0868	− 0.0509

Comparison of these numerical results suggests the following conclusions:

The mode, 5, is the same for the definite and indefinite on both sides, and for the total on each side it is 10. The averages also show little difference between the right and left jaws. From the variability, however, as indicated by the standard deviation, it appears that the number of teeth of the right jaw is slightly more variable than that of the left, σ being 0.074 greater on the right side for the definite teeth, 0.011 (which is less than the probable error) for the indefinite teeth, and 0.033 greater for the right total. The highest degree of variation is shown by the total number of teeth on the right side, and the least variation is shown by the number of indefinite teeth.

The form of the distribution curve of definite teeth on both jaws falls under Pearson's Type I, with the peculiar result of a slight negative skewness for the left side, and a positive skewness, although a very slight one, +0.0153, for the right. This indicates a tendency to the production of fewer definite teeth than normal on the left side, and a faint tendency towards a greater number on the right side. The distribution curve of indefinite teeth on both sides is of Type IV, with a negative skewness, −0.043, on the left side, and twice this, −0.086, on the right; *i.e.*, there is on the right side a greater tendency than on the left towards the production of few indefinite teeth. On the right side, then, it is clear that the probability of a large number of definite teeth is associated with that of a small number of indefinite teeth. The same thing is shown for the left side, for although definite and indefinite teeth both show negative skewness, the negativeness is much greater in the indefinite than in the definite teeth. Therefore, relatively, the skewness of the indefinite and the definite teeth may be said to be, here also, of opposite sense. This agrees with results shown in the correlation table, to be noted later.

A peculiar result is obtained in regard to the distribution curve of the total number of teeth. The left total falls into a curve of Type I, while the right total is of Type IV. The negative skewness of the latter is −0.050, while that of the former is about two and one-half times as much. The table of frequencies shows that the right total includes two classes more, one at each end of the series, than the left total. There is one individual in each of these two classes. It seemed probable, by inspection of the calculation, that the critical function, F, which was negative 0.5314, might be made positive by dropping these two extreme individuals, thus giving a curve of Type I. I found this to be the case, and obtained for F the value +0.210; but I found further that Type I might be obtained by dropping only the individual of Class 5, making F +0.0389. The skewness in this case was very slight, only −0.00706.

In order to determine which was the closer fit of the observed curve to the theoretical curve in the two types, I calculated the theoretical curves from the observed data with the following result.

TYPE IV.[1]

$n = 400$ $d = 0.06822$ $M = 10.055$
$s = 25.66$ $m = 13.830$ $y_0 = 96.34$
$a = 6.5798$ $\sigma = 1.3386$ zero ordinate $= 9.1114$ (M-md)
$v = 3.6796$ $\phi = 8^\circ\ 9'\ 7''$ $\tan\theta = {}^{x}/_{a}$
$y = y_0\,(\cos.\ \theta)^{2m}\, e^{-v\theta}$

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[1] For the methods of calculating the results given in the following tables, see Davenport, '99, pp. 20, 23, 24.

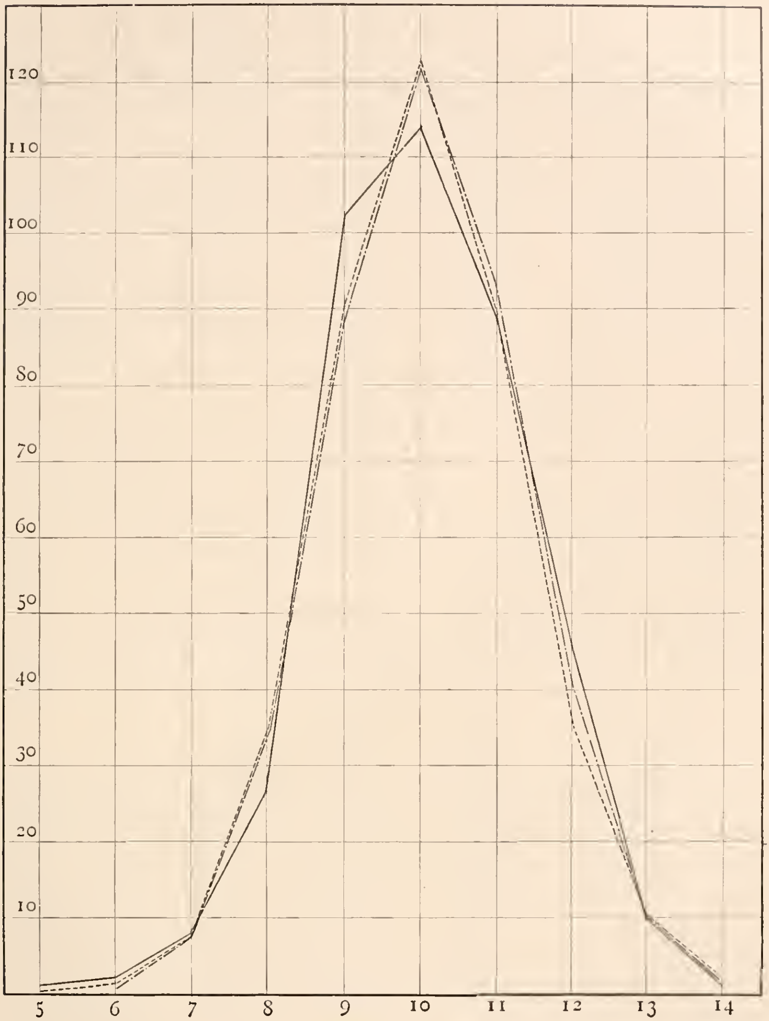

PLATE I.

——— Polygon of observed frequency.
········ Polygon of theoretical frequency, Type IV.
—·—·— Polygon of theoretical frequency, Type I.

Δ, Calculated by Duncker's method, = 3.68%.

$V-M$	f	y	δ_1	δ_2
− 4.1114	1	0.1	+ 0.87	
− 3.1114	2	1.2	+ 0.8	
− 2.1114	8	7.9	+ 0.1	
− 1.1114	27	35.3	− 8.3	− 0.09
− 0.1114	102	90.5	+ 1.5	− 4.82
+ 0.8886	114	122.9	− 8.9	− 5.01
+ 1.8886	89	89.9	− 0.98	
+ 2.8886	46	35.5	+ 7.43	− 0.85
+ 3.8886	10	10.8	− 0.89	− 0.79
+ 4.8886	1	2.3	− 1.36	
	400	399.7	41.05	11.56

TYPE I.

$n = 399$ $s = 305.56$ $b = 46.165$
$a_1 = 21.663$ $m_1 = 142.426$ $\sigma = 1.316$
$a_2 = 24.502$ $m_2 = 161.134$ $d = 0.00929$
$y_0 = 120.68$ (calculated from approximate formula). $M - d = 10.066$

$$y = y_0 \left(1 + \frac{x}{a_1}\right)^{m_1} \left(1 - \frac{x}{a_2}\right)^{m_2}$$

Δ, Calculated by Duncker's method, = 3.58%.

$V-M$	f	y	δ_1	δ_2
− 4.066	2	0.9	+ 1.07	
− 3.066	8	7.8	+ 0.2	
− 2.066	27	35.2	− 8.24	− 0.19
− 1.066	102	87.2	+ 14.84	− 5.29
− 0.066	114	121.5	− 7.46	− 4.96
+ 0.934	89	93.1	− 4.08	
+ 1.934	46	41.1	+ 5.06	− 2.25
+ 2.934	10	10.3	− 0.3	− 0.29
+ 3.934	1	1.4	− 0.42	
	399	398.5	41.60	12.98

Since it is considered a sufficient agreement between observation and calculation when $\Delta < \frac{100}{\sqrt{n}}\%$, which in this case is 5%, it is evident that these values show a moderate degree of closeness of fit of the two curves. The difference between the two values of Δ, 0.10, is so small that we may conclude that it is practically immaterial under which type this curve should fall.

The fact that by dropping one individual from a series a curve may be thrown from Type IV to Type I and may be made to fit equally well in either case, raises a serious question as to the biological importance of the distinction between Pearson's Type I and Type IV. Pearson ('**95**) himself says: "It seems very possible that discreteness rather than continuity is characteristic of the ultimate elements of variation; in other words, if we replaced the curve by a discrete series of points, we should find a limited range. It is the analytical transition from this series to a closely fitting curve which replaces the limited by an unlimited range. Exactly the same transition occurs when we pass from symmetrical point binomial to normal curve. Thus while Type I marks an absolutely limited range, Type IV does not necessarily mean that the range is actually unlimited."

It appears from the results obtained in the two calculations given above that even less value can be placed upon any distinction between Type I and Type IV than is suggested by Pearson. The difference of one individual actually causes, here, the transition from one type to the other, the individual being at the extreme of the series.

3. *Correlation.*

In the table on the following page every possible combination of teeth for the two sides is given, together with the actual number of specimens for each combination of definite, indefinite, and total teeth.

From this series of combinations the following results were obtained for the coefficient of correlation. The calculations were made by Pearson's method and checked by the briefer method of Duncker.

Correlation between Right and Left Definite Teeth, $r = +0.688 \pm 0.0136$
" " " " " Indefinite " $r = +0.725 \pm 0.0121$
" " " " " Total " $r = +0.820 \pm 0.0081$
" " Right Definite and Left Indefinite, $r = -0.424 \pm 0.0231$

Bearing in mind that an index of 1 signifies a perfect correlation, and that 0 indicates an entire lack of it, we see that the

III. Correlation Table.

Teeth.		Specimens with definite teeth.	Specimens with indefinite teeth.	Teeth.		Specimens with definite teeth.	Specimens with indefinite teeth.	Teeth.		Specimens with total teeth.	Teeth.		Specimens with total teeth.
R.	L.			R.	L.			R.	L.		R.	L.	
1	2		2	5	5	67	79	5	6	1	11	9	1
1	4	1		5	6	26	20	6	6	2	11	10	20
2	2	2	5	5	7	6	2	7	7	7	11	11	60
2	3	1	2	5	8		1	7	8	1	11	12	7
2	4	1		6	3	2		8	7	4	11	13	1
3	2	2	4	6	4	8	5	8	8	13	12	9	2
3	3	14	12	6	5	17	32	8	9	8	12	10	5
3	4	14	11	6	6	49	54	8	10	2	12	11	14
3	5	6	2	6	7	9	7	9	8	11	12	12	23
3	6		1	7	4	1		9	9	61	12	13	2
3	7	1		7	5	4	5	9	10	25	13	12	6
4	2	1	1	7	6	13	15	9	11	5	13	13	4
4	3	7	9	7	7	21	8	10	7	1	14	12	1
4	4	41	39	7	8	2		10	8	3			
4	5	36	27	7	9		1	10	9	23			
4	6	9	4	8	6	3		10	10	64			
5	3	6	8	8	7	5	3	10	11	21			
5	4	22	38	8	8	3	3	10	12	2			
						400	400						400

degree of correlation between the right and left sides is, on the whole, rather high. It seemed at first a somewhat unexpected result that the correlation in the variability of what I have called the indefinite teeth should be higher than in that of the definite teeth. If the correlation had been perfect it would have meant that those causes which produced a deviation from the mean in the left sets acted in the same degree on the right sets also. Is it possible then that different causes may have acted or that the same cause may have been effective in different degrees in producing the variability in the definite and the indefinite teeth?

This question drew my attention more closely to a fact noticed only incidentally in counting the teeth, *i.e.*, in case of animals having dark, heavy jaws, evidently older animals, the definite teeth were fewer in number than in case of small, young individuals. In the older jaws the teeth began farther from the tip, leaving a smooth point, while the younger, more delicate jaws were often finely denticulated to the extremity. The serratures of the older jaws appeared to be worn off by use. In order to determine whether or no the correlation actually existed between the size or age of an animal and the number of definite teeth, I made the comparison for one hundred individuals, taking as an indication of size, and hence roughly of age, the head length in millimeters. This was measured from the anterior edge of the first ring to the extremities of the two apical feelers. The result was a negative correlation, although a rather small one, -0.128. It is probable, then, that age does come in as a factor in the production of a small number of teeth, and that this decrease is due to wear. It is possible also that the wear may be heavier upon one jaw than upon the other, thus accounting for the comparatively slightly lower degree of correlation between the definite teeth than between the indefinite teeth. For wearing does not act at all directly on the indefinite teeth, since they do not emerge from the chitinous covering, and are often sunk in the tissue of the proboscis. It would be interesting to know in what manner the jaws are carried and work upon each other during the life of the animal, for a certain habit of crossing them might also account for the peculiar differences in skewness of the curves of the right and left teeth noted in the discussion of constants. The smallness of the negative index of correlation between the age of an animal and the number of definite teeth shows that age does not play a very important part in causing variation.

An attempt to correlate the number of definite and the number of indefinite teeth on the right jaw resulted in a negative index of correlation, -0.424. This fact indicates an inverse relation between the definite and indefinite teeth on the same jaw; that is, a jaw with a small number of definite teeth will probably have a comparatively large number of indefinite teeth, and inversely. It may be that indefinite teeth continue to be laid down at the base of the jaw during the life of the animal, in which case the number would tend to be greater with age, while, as we have seen, the number of definite teeth is smaller.

4. *Relation of Individual and Specific Variation.*

Out of fifty different species of Nereis which I found described by various authors, the number of teeth was stated for forty-seven. The numbers ranged from 0 in two cases, in which the edentalous condition of the jaws was an important specific character, to 20, given by Audouin and Milne-Edwards ('29) for *N. fucata.* The number of teeth of *N. fucata* is given by Ehlers ('68), however, as 7, by Johnston ('65) as 5 to 10, and in the Challenger ('85) reports as 14 to 16. It would be difficult here, as in the case of a few others, to decide which observer came nearest to the modal condition of the species. It is also impossible to tell whether they counted the total number of teeth including those covered by a chitinous band, or whether they referred only to the prominent definite teeth. Ehlers makes the distinction only in *N. virens*, where he gives the definite teeth as 5 to 6, total as 10. St. Joseph ('88, '98) notes in description of *N. diversicolor* that of 8 teeth 2 are indefinite, and in *N. floridana* that of 9 teeth the lower 4 are buried in a clear translucent covering. For these two species, respectively, Ehlers has given 8 and 9 teeth, evidently counting both definite and indefinite.

After attempting various methods of striking averages of the statements made by different authors I finally decided to use Ehlers's numbers alone as most reliable, adding a few of those given by St. Joseph in which there was less doubt that the total number of teeth had been counted. Seriation of twenty-two species gave the following results, total teeth.

Classes . . .	5	6	7	8	9	10	11	12
Frequencies	1	1	5	2	6	3	2	2

CONSTANTS.

A = 8.727

$\sigma = 1.838$

$\beta_1 = 0.000090$

$\beta_2 = 2.323$

F = + 1.354

curve = Type I

s = 5.862

skewness = + 0.00966

Any conclusions which can be drawn from these results are necessarily of doubtful value. It will be seen that the mean for the number of teeth in twenty-two species is lower than the mean of total teeth in the one species which I have described. The skewness of the curve instead of being negative is positive, although it is exceeding small. Had it been negative, as I had thought it might be, it would have indicated that in the species of the genus, as well as in the individuals of the species, there had been a movement in the direction of a smaller number of teeth, either from an excessive production of individuals and species having few teeth, or from selective annihilation of those having many teeth. The opposed positive skewness is so small that it may mean little in regard to the species, and particularly since the numbers are small and the method of counting so doubtful no stress can be laid upon it.

5. *Abnormalities.*

Differences between the two jaws of the same animal in the definite, indefinite, or total number of teeth were of common occurrence. The accompanying drawings are intended to show some irregularities of this kind. In Fig. 1 the right jaw has four large definite teeth and five below which do not emerge from the surrounding chitinous layer; the left jaw has only slight crenulations corresponding to five definite teeth, although it has the same number of indefinite teeth as the right side. Fig. 2 shows on the right jaw three definite teeth, the edge above and distal to them having three very slight elevations; the opposite jaw ends in a long point with a perfectly smooth edge and has only two large definite teeth below. There is the same number of indefinite teeth on both sides. Figs. 3 and 4 show variations of the same kind, the numbers of both definite and indefinite teeth being different for the two jaws.

So far the drawings have been made from old animals in which the jaws are hard, strong, and very dark in color. It is probable from the appearance of the jaws that the difference in the number of definite teeth is due largely to the wearing, on one side or the other, of the distal teeth. Fig. 5 shows a common irregularity of equal totals with slight differences in the combinations of definite and indefinite teeth, the left jaw having 8 to 4, the right 6 to 6. Figs. 5 and 6 are from small, young animals, and the jaws are seen to be more slender with numerous fine teeth, 13 on the right and 12 on the left jaw.

The specimen drawn in Fig. 7 was interesting in regard to the indefinite teeth. The left jaw presented the usual appearance of six indefinite teeth placed fairly regularly and six definite teeth. On the right jaw the point was worn smooth, leaving only four definite teeth, while below three normal indefinite teeth was a series of five small ones placed very close together instead of three as on the opposite jaw. This may indicate a tendency towards regulation by the production

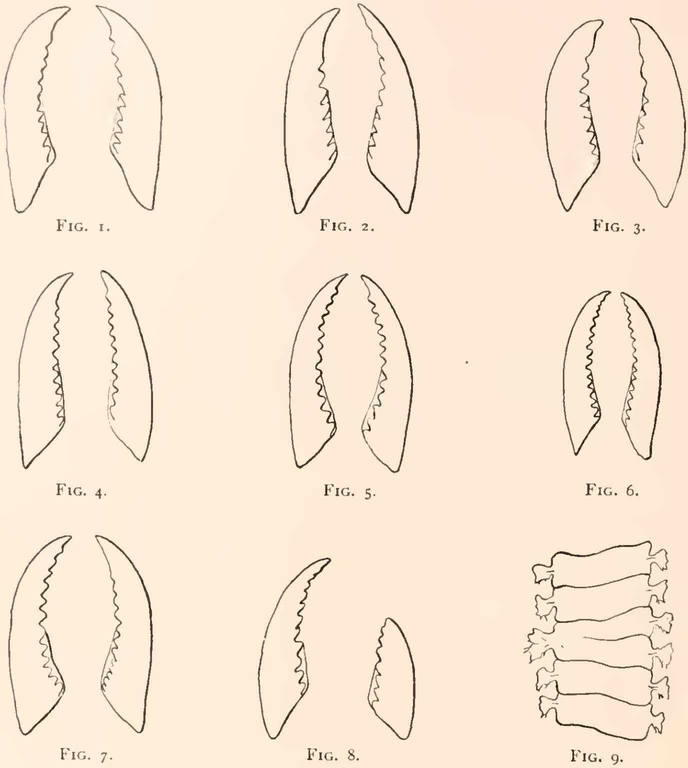

Fig. 1. Fig. 2. Fig. 3.

Fig. 4. Fig. 5. Fig. 6.

Fig. 7. Fig. 8. Fig. 9.

Plate II.

Figs. 1–8. — Variation and abnormalities in teeth on opposite jaws.
Fig. 9. — Abnormal segment.

of an excess of teeth at the base of a jaw on which some of the extreme teeth had been lost, but I found no other indication of such regulation. Another individual presented a partial right jaw, Fig. 8, which was a stump of about half the length of the left jaw. The normal jaw was dark brown, almost black, while the stump was light straw color characteristic of a young jaw or of the very base or imbedded part of an old one. The color indicated new growth or regeneration, in which case one would expect to find a production of small indefinite teeth crowded at the base, as in the specimen of Fig. 7, if that could be interpreted as a regenerative process. On the contrary, the stump had exactly the same number of teeth similarly disposed as the part of the opposite jaw which corresponded to it. It may have been, then, only the rounded stump of a broken jaw, although this explanation does not account for the peculiar color.

Abnormalities in other parts of the animals were looked for only incidentally. I found no cases of double pairs of caudal cirri, but all of the worms were not examined for this not unusual abnormality, since the posterior parts of many of them were not preserved.

Fig. 9 shows a case of an abnormal segment. The fifteenth segment extended only a little more than halfway across towards the left side of the body, leaving one broad segment on the left side in place of two, and a partially double parapod. The axis of the body was bent at that point, compensation being made gradually by the greater width on the left side of the segments immediately preceding and following.

6. *Summary.*

The results of this study may now be summed up as follows:

(1) The typical condition for the total number of teeth of *N. limbata* of Cold Spring Harbor, 1899, is a curve of either Type I or Type IV, with a slight skewness in a negative direction from the mode, 10.

(2) In case of the calculation of the right total teeth, a transition from a curve of Type IV to an equally serviceable

one of Type I could be made by discarding one extreme individual out of four hundred.

(3) The number of teeth on the right jaw appears to be slightly more variable than that on the left.

(4) The degree of correlation between the two jaws is, on the whole, rather high, 0.820. Correlation is closer between the indefinite than between the definite teeth. An inverse relation exists between the number of definite and the number of indefinite teeth on the same jaw, and also one between the number of definite teeth and the age of an animal.

(5) The class range of teeth as given by the different species of the genus Nereis has a close agreement with the class range of *N. limbata*. Hence this one species offers the material for the modal condition of all species of the genus.

(6) The results of observations of many specimens showing irregularities in teeth point to the conclusion that a process of wearing away of the definite teeth takes place in mature animals, and therefore that age comes in to help produce small number of teeth, but is not a large factor in causing variation.

Only one author, St. Joseph, makes note of a difference between young and old specimens, the young having the greater number of teeth. Thus the statements made in regard to the number in many species in which only one animal or at most very few specimens were seen and described by their discoverers, are of little value as criterions of the specific condition.

In conclusion, I wish to express my thanks to Professor Charles B. Davenport, who not only generously furnished the material for this investigation, but by his oversight and advice greatly facilitated the progress of the work.

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BIOLOGICAL BULLETIN.

THE CENTROSOME IN THE MATURATION AND FERTILIZATION OF BULLA SOLITARIA.

MARTIN SMALLWOOD.

THE material upon which the following observations were made was collected at Woods Holl during the seasons of 1898–1900. The greater part of the work was done at the Marine Biological Laboratory under the direction of Dr. E. G. Conklin, and I take this opportunity of thanking him for his many valuable suggestions. I also wish to acknowledge my indebtedness to Dr. C. O. Whitman and Dr. C. W. Hargitt.

A fuller account of my observations both upon the subject of this paper and the cell lineage of Bulla, with a discussion of the pertinent literature, will be published later.

The sketches illustrating the mitotic changes in maturation were drawn from sections stained with Heidenhain's iron-alum followed by an aqueous solution of Bordeaux. In order to differentiate the sperm, it was necessary to use Conklin's mixture of haematoxylin and picric acid. The sperm, therefore, has been drawn from corresponding stages and inserted into these figures.

In the interpretation and nomenclature of the centrosome and sphere I have followed in the main Van Beneden. The term "centrosome" will be applied to the body which occurs at the pole of the spindle, etc., when that body has become differentiated into a central corpuscle, surrounded by a clear area, the medullary zone bounded by a definite wall. The body occurring at the center of the aster is the central corpuscle.

The central corpuscle becomes the centrosome. Sections of the ovotestis before copulation show the unfertilized egg lying free in the follicles of the hermaphroditic gland. The large germinal vesicle lies in the center of the egg; it contains a large vacuolated nucleolus, also basichromatin and oxychromatin granules. The deutoplasmic spheres are equally distributed in the cytoplasm and conceal its structure. I have not been able to discover any evidence of a central corpuscle or centrosome in the egg before mitosis begins. By the time the eggs are laid the first polar spindle is in the end of the prophase. In order to secure the earlier stages, a large number of animals were collected and killed as soon as they began to lay. The first polar spindle begins to form as the animals begin to lay. Sections of the ovotestis taken from animals killed while they were laying revealed the fact that every mature egg had already passed through the early prophase of the first spindle; even those eggs in the most distant follicles, where it is probable that the sperms from the receptaculum seminalis had not penetrated; I have found the sperms in the anterior part of the hermaphroditic duct, but not extending back to any considerable distance. The earliest stage thus far discovered had two well-formed central corpuscles and a definite central spindle connecting each, which passed through the germinal vesicle, the walls of which are plainly visible. The ring-shaped chromosomes have begun to form from the meshwork of linin and chromatin. The reduction of the chromosomes has not been worked out in detail. These ring-shaped chromosomes gradually take a deeper stain and come to lie in the equatorial plate of the first polar spindle (Fig. 1). In a cross-section of the equatorial plate I was able to count sixteen distinct chromosomes. There is a distinct cortical zone surrounding the central corpuscle. The astral rays pass through this clear area and extend to the central corpuscle. At this stage I have been able to

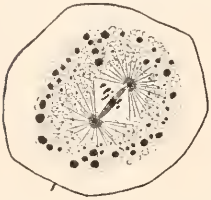

FIG. 1. — Taken from the ovotestis. Shows the central corpuscle surrounded by the cortical zone of the sphere. The central spindle is well formed and the chromosomes are forming into the first equatorial plate. Walls of the germinal vesicle still present, sperm entering at vegetal pole.

trace them nearly to the egg membrane. From now on, there does not appear to be any appreciable change in the mitotic figure, until after the egg has been laid, when it begins to migrate to the periphery of the egg, where it assumes a radial position. There is no difference in the character of the two poles of the spindle. During this movement of the spindle the chromosomes pass into the metaphase, and the centrosome becomes differentiated into a central corpuscle and a medullary zone which is limited by the walls of the old central corpuscle. This is the first time that we have a structure to which we can apply the term "centrosome" in the sense that I purpose to use the term. The cortical zone has enlarged and become much fainter. The chromosomes do not divide at once; the activity is centered in the centrosomes. While the chromosomes are still in the equatorial plate, the central corpuscle in each centrosome divides, having the dumbbell form. The centrosome rapidly increases in size, the periphery is limited by a definite line, which gradually becomes thinner. The medullary zone does not take a plasma stain, as it did in the previous stage (Fig. 9). The centrosome now begins to assume an elliptical form and at the same time to rotate. This rotation continues until the elongated centrosome, which encloses the second polar spindle, lies radially and in the same position that the first polar spindle did. The central corpuscles, connected by a central spindle, are so influenced by this elongation of the walls of the centrosome that they come to lie near the ends — at the foci of the ellipse. As the outer pole of the second polar spindle nears the periphery of the egg, the rays extend to the chromosomes, and they are pulled into the spindle to form the equatorial plate of the second polar spindle. It will be seen that in the main my results corroborate those of MacFarland,[1] Lillie,[2] and Conklin.[3]

The changes which take place in the centrosome during this stage are very interesting. The centrosome is so large and comes out with such perfect clearness that I have been able to follow the details very carefully. The spindle is developed when the central corpuscles separate. At this time a very faint line can be seen connecting the new corpuscles; as the distance between them increases, the line becomes more distinct, until a central spindle can be clearly distinguished. In the mean time the line limiting the centrosome has become broken into pieces, which gradually become smaller and smaller until they cannot be distinguished from the granules of the cytoplasm. While these changes have been taking place, this broken line has served to mark the outer limit of the medullary zone. The old medullary zone has disappeared, and between the central corpuscle and the broken wall of the centrosome we have a new medullary zone, which is the cortical zone of the second polar spindle. The process is as follows: as the walls of the centrosome begin to break down, an area next to the central corpuscles and at each end of the centrosome appears; this begins to take a plasma stain (Fig. 3). The area gradually surrounds the central corpuscle and all of the space at the end of the spindle between the central corpuscle and the wall of the centrosome. In the mean time the central corpuscle has increased in size and is to become the centrosome of the second polar spindle; it ultimately becomes differentiated into a central corpuscle and a medullary zone.

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[1] "Celluläre Studien an Mollusken-Eiern," *Zoöl. Jahrb.* 1897.

[2] "Centrosome and Sphere in the Egg of Unio," *Zoöl. Bull.* Vol. i, No. 6. 1898.

[3] *Science*, March, 1898.

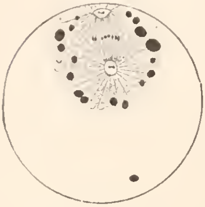

FIG. 2. — Metaphase of first maturation spindle. Two central corpuscles at each pole. Cortical zone limited by a dotted outline. The medullary zone does not take a plasma stain at this stage. Sperm head solid and elliptical.

Fig. 2 shows two central corpuscles in the centrosome at each pole of the spindle. Linville[1] shows a similar but not identical stage. The centrosome of the outer pole of the spindle does not enlarge more than is shown in the figure; as it reaches the surface of the egg, it breaks down and the central corpuscles form the division centers in the first polar body.

There is no telaphase in the first maturation spindle. The nearest approach to such a stage is shown in Fig. 3, where the chromosomes have become partly hollow vesicles. A few of the interzonal fibers show at this stage, but they are very faint.

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[1] "Maturation and Fertilization in Pulmonate Gasteropods," *Bull. Mus. Comp. Zoöl., Harvard.* Vol. xxxv, No. 8.

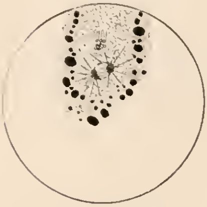

Fig. 3. — Prophase second maturation spindle. Wall of centrosome broken in pieces. Central corpuscles connected by a spindle. New cortical zone forming. Chromosomes are partly hollow. A few interzonal fibers are present.

In the metamorphoses of the centrosome its attachment to the astral rays is plainly evident; the old rays can sometimes be seen in a stage younger than the one shown in Fig. 11, when the new rays have already begun to form and are attached to the central corpuscle. I believe that the rays of the first polar spindle disappear and that the rays of the second spindle rise *de novo*.

The metakinesis of the second polar spindle takes place very rapidly. The chromosomes elongate, divide transversely, and as they move toward the poles, they assume a roundish form, and change into vesicular bodies which fuse to form the female pronucleus. During the time when they are fusing, the rays can be traced directly into the areas immediately surrounding them. In the stage of anaphase as represented in Fig. 5, the centrosome is evident, although it does not stain as deeply as in Fig. 4. Immediately after this stage the centrosome disappears and the cortical zone enlarges and completely surrounds the female pronucleus; later both male and female pronuclei come to lie in this clear area. A single centrosome passes off with the second polar body, which is much smaller than the one given off in the first polar body (Fig. 4).

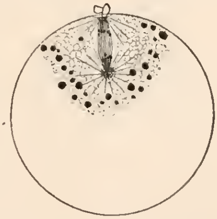

Fig. 4. — Anaphase second maturation spindle. Centrosome at each pole with a single central corpuscle. The medullary zone takes a plasma stain.

The eggs of this species are not especially favorable for a study of the problem of fertilization. During all of the earlier stages, the sperm head lies completely surrounded by deutoplasmic spheres. I have not been able to make out any continuous clear area about the sperm head during its progress through the egg. In one instance there was a definite clear area about the sperm nucleus after it had nearly approached the female pronucleus, otherwise it was unattended by anything that corresponds to the "Hellerhof" of MacFarland and others. The sperm enters at the vegetal pole, though not in any definite place. The tail is lost before the sperm enters the egg membrane (Fig. 1). The head is a solid body having a distinct angle in the middle. If there is a middle-piece, it is practically indistinguishable. The only indication that I have found of such a body is that on one end of the sperm head sometimes one finds a narrow band that stains a little denser than the rest of the head.

The sperm head becomes top-shaped as it begins to migrate toward the animal pole with the point leading. The head becomes elliptical (Fig. 3) and stains very black. It remains in this solid form for some time, while the first polar spindle is passing from the metaphase until the anaphase of the second polar spindle. During the anaphase of the second maturation, the solid sperm head becomes vesicular, and for a very short time is accompanied by astral rays. I have not been able to discover a central corpuscle in connection with the aster, nor have I ever seen an amphiaster. At this same time secondary asters usually appear in the egg, which are smaller than the sperm aster. As the chromosomes of the second polar spindle begin to assume the vesicular form, the sperm aster disappears, and the sperm, consisting of one or more vesicles, rapidly approaches the inner pole of the second polar spindle. When the sperm consists of more than one vesicle, these fuse into one when the aster disappears. While the vesicular sperm is shifting its position it does not increase in size to any noticeable extent, but as soon as it comes near the female pronucleus, which now consists of but three or four vesicles, it rapidly increases in size until it is about twice as large as it was when migrating toward the animal pole. From the time that the sperm head enters the egg until it comes to lie in contact with the female pronucleus (Fig. 6), it is not attended, so far as I have observed, by any body which might be taken for a central corpuscle or a centrosome.

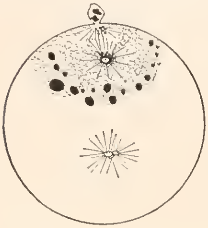

FIG. 5. — Late anaphase of second maturation spindle. Centrosome still present. Cortical zone enlarging. The sperm is composed of three vesicles and accompanied by astral rays.

The structure of the two pronuclei when they have come together (Fig. 6) is the same. The male pronucleus is usually regular in outline and slightly smaller. The irregularities in the outline of the female pronucleus often persist until the central corpuscles of the first cleavage appear. The chromatin stains very slightly and is connected by delicate linin threads. The changes through which the chromatin passes before the equatorial plate is formed may be described under three stages. First, the chromatin rapidly increases in staining power, forming a dense reticulum, often containing stellate masses of solid chromatin. Second, the chromatin begins to assume a definite form. The most conspicuous shape is the stage where the masses of chromatin have begun to break up into rings but are still attached to one another. The chromosomes have not yet become hollow. They stain uniformly. Third, the chromosomes have broken apart from each other, and have become hollow, round bodies. At first there is a delicate meshwork connecting them (Fig. 7), but this is soon lost and each pronucleus is filled with ring-shaped chromosomes. While the chromatin is undergoing the last two changes, the central corpuscles (the so-called cleavage centrosomes) of the first cleavage spindle make their first appearance. I have found them in a much earlier stage than the one figured, but in each case there was no connection between them; but these corpuscles with their rays have a definite relation with the pronuclei, that is to say, each pronucleus has an aster and central corpuscle with a faint cortical zone. My observations upon the cleavage centrosomes would tend toward the position, first, that they arise *de novo*; and, second, that one arises in connection with each pronucleus.

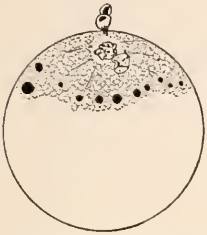

Fig. 6. — The female pronucleus is irregular in outline, surrounded by the cortical zone of the inner pole of the second maturation spindle. A few astral rays are present. Zwischenkörper of second polar body present.

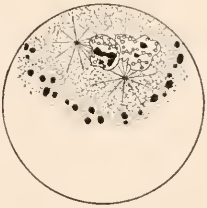

Fig. 7. — Origin of cleavage centrosomes. One in connection with each pronucleus. The central corpuscles are surrounded by a faint cortical zone.

Metamorphosis of the Centrosome in Maturation.

The definiteness and clearness with which the several changes in the centrosome appear in Bulla make these changes the most important of the various stages in maturation and fertilization. In describing the changes of the centrosome, under various stages, I have no theoretical points in consideration. While the stages figured are clearly differentiated, still there are intermediate stages which graduate imperceptibly into one another.

In the earliest prophase that I found the central corpuscle was a large solid mass (Fig. 8). Surrounding the central corpuscle there was a conspicuous area, the cortical zone, which was sharply differentiated from the cytoplasm. The rays are not lost in the cortical zone, as MacFarland has shown for Diaulula, but extend to the central corpuscles, as Lillie has shown for Unio, and Linville for Limnaea. However, I do not find a row of microsomes, as in Unio, limiting the sphere, nor is the boundary formed by the fusing of the astral rays, as in Limnaea.

Second stage (Fig. 9). The central corpuscle has become clearly differentiated into a centrosome. It reacts to stain in a very different manner from what it did in a previous stage. There is now a medullary zone which takes on a plasma stain and is limited by a distinct line. The small dark body in the center is the new central corpuscle. The cortical zone has increased in size and is less easily distinguished from the surrounding cytoplasm. The line marking the periphery of the centrosome is the limiting wall of the enlarged central corpuscle of the previous stage.

Third stage (Fig. 10). The centrosome has increased in size. The line at the periphery is definite and whole. The central corpuscle of the previous stage has divided into two central corpuscles, which are connected from the first by faint lines. The medullary zone does not take a plasma stain. The cortical zone has become very faint and soon disappears as a distinguishable area in the cytoplasm.

Fourth stage (Figs. 3 and 11). The periphery of the centrosome loses its continuity, and openings occur in the wall; while these changes in the wall are taking place, the centrosome becomes much enlarged and assumes an elliptical shape. Immediately after these breaks appear, there is a small part of the medullary area which takes a plasma stain. This area is somewhat triangular in shape and occurs at the end of the centrosome between the central corpuscle and the broken periphery of the centrosome. New astral rays are formed which extend to the central corpuscle. The old astral rays can be seen disappearing at this stage in the cytoplasm.

Fifth stage (Fig. 12). The central corpuscle of the second polar spindle has enlarged and is still undifferentiated. The pieces of the periphery of the old centrosome have become smaller. There is now a distinct cortical zone around the central corpuscle, which has been derived from the medullary zone of the centrosome of the first polar spindle. This stage is identical with the first one described, except that the rim of the old centrosome is still present and the central corpuscle is only about one-half as large.

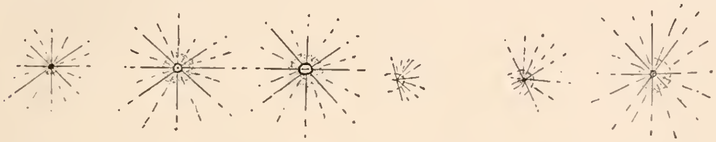
FIG. 8. FIG. 9. FIG. 10. FIG. 11. FIG. 12. FIG. 13.

FIGS. 8–13. — The changes through which the central corpuscle and centrosome pass from the prophase of the first maturation spindle to the metaphase of the second maturation spindle. In each case the solid dark body is the central corpuscle. The granular area surrounding the central corpuscle is the cortical zone. Figs. 9, 10, and 13 show a complete centrosome, having a central corpuscle and medullary zone.

The sixth stage (Fig. 13) shows the rim of the old centrosome still present, but in smaller pieces which do not stain as deeply as in the previous stage. The cortical zone has enlarged and become fainter. The centrosome is composed of a medullary zone and a central corpuscle.

Summary.

The central corpuscle of the first polar spindle becomes the centrosome of the second polar spindle with a medullary zone and a central corpuscle. The medullary zone of the centrosome of the first polar spindle gives rise to the cortical zone of the second polar spindle. The central corpuscle of the centrosome of the first polar spindle gives rise to the centrosome of the second polar spindle. Thus we may say that the centrosome of the first polar spindle in Bulla solitaria not only gives rise to the centrosome and mitotic figure of the second polar spindle, but to the cortical zone or outer sphere substance surrounding each centrosome.

Allegheny College,
October, 1900.

CONTRIBUTIONS ON THE MORPHOLOGY OF THE ACTINOZOA.

J. PLAYFAIR McMURRICH.

VI. HALCURIAS PILATUS AND ENDOCOELACTIS.

In 1892 Carlgren showed that certain Edwardsiae, whose tentacles were more numerous than the mesenteries, had these tentacles arranged on the hexactinian plan, their arrangement in this presumably primitive group of the Actiniaria seeming to foreshadow what is characteristic of the phylogenetically later group. In other multitentaculate Edwardsiae he found what seemed to be an octamerous arrangement combined to a certain extent with hexamerism, but later studies (**'99**) convinced him that the octamerism did not occur, and that in all cases the hexamerous arrangement obtained.

In the mean time an important discovery had been made by Faurot (**'95**) in studying *Edwardsia beautempsi* and *E. adenensis*, the former of which possesses fourteen to sixteen tentacles, while for the latter the number is stated to be fifteen to sixteen. Sections through the column showed the eight mesenteries, which have long been supposed to be the only mesenteries developed in the Edwardsiae; but in the uppermost portions a number of additional very short and narrow mesenteries were found which in *E. beautempsi* were placed in such a way as to make with the perfect mesenteries an arrangement recalling what occurs in *Gonactinia prolifera*. Thus there were eight pairs of mesenteries present in the upper part of the column, two of which, the directives, were formed of two perfect mesenteries, four of one perfect and one imperfect mesentery, and one of two imperfect mesenteries. In *E. adenensis* the additional short mesenteries were arranged in pairs in each interval between adjacent perfect mesenteries, except in the endocoel of the directives, so that in this form the arrangement differed somewhat from that typical for the hexactinians.

These observations show reason for believing that in the Edwardsiae there is an intimate relation between the number of tentacles and that of the mesenteries, and that when there are more than eight tentacles there is a strong probability that a number of short mesenteries are also present in the upper part of the column. It is a general rule in the Actininae that the number of tentacles in the fully developed condition is double that of the pairs of mesenteries or, in other words, that there is a tentacle corresponding to each endocoel and each exocoel, the number of tentacles being equal to the total number of mesenteries. Exceptions, due to a lack of development of the full complement of tentacles, are of common occurrence, in many cases probably owing to the specimens examined not having reached their full development, though even in some adults, apparently, the number of tentacles never reaches that of the mesenteries, as is the case, for instance, in *Peachia hastata*, which, with twenty mesenteries, never has more than twelve tentacles.

The rule may be better expressed by saying that *the number of the tentacles never exceeds that of the mesenteries*, and when an apparent exception to this occurs the presumption is that closer examination will reveal the existence of small mesenteries limited to the upper part of the column and in sufficient numbers to fulfill the requirements of the rule.

Acting on this supposition, I have made a further study of the upper portion of the column of *Halcurias pilatus*, a form which I have already described as possessing twenty mesenteries and a number of tentacles considerably in excess of that of the mesenteries, having been estimated in one specimen ('93) to be about seventy, and in another ('98) to be about sixty. Sections showed, as I had expected them to do, the presence of a number of short and narrow mesenteries in the upper part of the column, the number of these *plus* the twenty perfect mesenteries being equal to the total number of the tentacles, which proved to be sixty-eight.

The sections also revealed, however, a peculiarity which I had not expected, and which, as may be seen from Fig. 1, consisted in the short, narrow mesenteries being developed in the endocoelic spaces bounded by the perfect mesenteries. The sections did not, unfortunately, cut the column perfectly transversely, but the arrangement which obtained may be perceived from the representation of the half of one section shown in Fig. 1, and from the diagram (Fig. 2) which represents a reconstruction from a perfect series of sections. On each side of the median line of Fig. 1 is one of a pair of directives, that to the right being cut at the level of the oral stoma, as is also another mesentery in the right half of the figure. On each side of the directives is a perfect mesentery with its muscle pennon on the same side as that of the adjacent directive, and on the left side this mesentery is succeeded by one which evidently forms with it a typical pair, though it may be noticed that the endocoel enclosed by this pair is broader than the adjacent exocoels. On the right side, where, owing to the obliquity of the sections, the column is cut higher up, the bases of some of the tentacles (T) being cut, the mesenteries of the first lateral pair are widely separated, and between them three imperfect pairs occur, which evidently represent two cycles. The succeeding

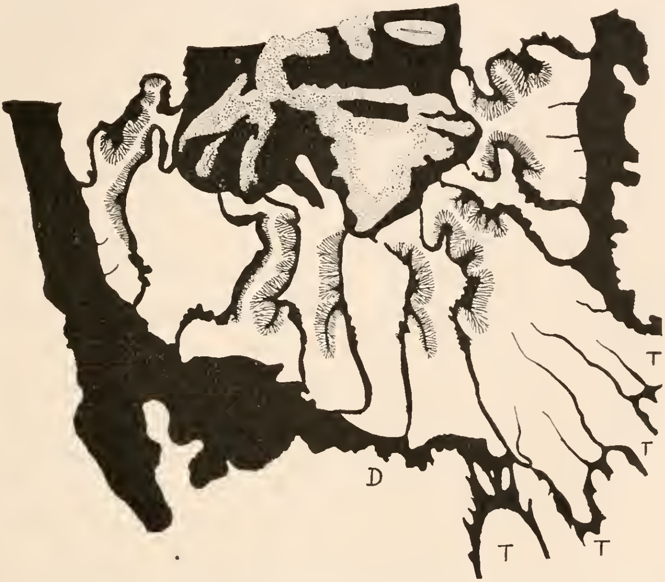

Fig. 1. — Transverse section through the upper part of the column of *Halcurias pilatus*. T = tentacle. D = directive mesenteries.

exocoel is much narrower than either of the adjacent endocoels and contains no imperfect mesenteries, while in the next endocoel two pairs of mesenteries are seen on the right side and one on the left. By following through the series of sections it is readily seen that the arrangement found in the first lateral endocoel of the right side is repeated in all the others, except in the cases of the endocoels enclosed by the directives, and the condition represented diagrammatically in Fig. 2 is that which obtains.

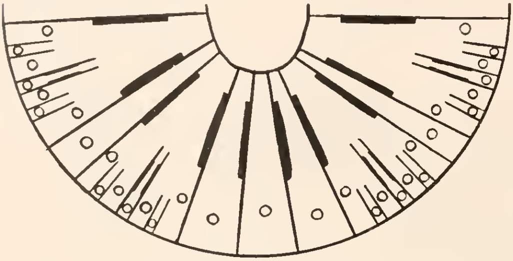

FIG. 2. — Diagram showing the arrangement of the mesenteries and tentacles in *Halcurias pilatus*.

Owing to the relative widths of the endocoels and exocoels, and the presence of imperfect mesenteries in the former, the first impression one receives is that of a form with a large number of directive mesenteries. That such an interpretation of the conditions is erroneous is clearly shown, however, by reference to the mesenteries on either side of the true directives. It is interesting to note that the development of the imperfect mesenteries, which are plainly arranged in two cycles, follows the hexactinian rule, the smaller pairs being developed in the intervals between the larger pairs and the adjacent perfect mesenteries. It may be added that my sections show the existence of a marginal stoma in each perfect mesentery in addition to the oral stoma already mentioned.

From the description given above, it will be perceived that the arrangement of the mesenteries in *Halcurias pilatus* is identical with that described by Carlgren ('**97**) for a form from the Chinese seas which he refers to the genus *Endocoelactis* and to a new family, the Endocoelactidae. The similarity to *Halcurias* is by no means confined, however, to the arrangement of the mesenteries, and there can be no question but that the two forms must be referred to the same genus, to which, notwithstanding the greater appropriateness of Carlgren's name, the prior term, *Halcurias*, must be applied. The specific identity of Carlgren's form with *H. pilatus* seems improbable; for, apart from the difference in the localities for which the two have been obtained, the tentacles of the Chinese form are longer apparently than those of *H. pilatus*, and to judge from Carlgren's figures, the longitudinal musculature of the tentacles is weaker and its mesogloeal processes coarser. It seems preferable at present to regard them as distinct, and since Carlgren, in his brief notice, has bestowed no specific name on his *Endocoelactis*, I would suggest that it be named *Halcurias Carlgreni*, as a slight recognition of the admirable work which that author has accomplished on the morphology of the Actiniaria.

An examination of the arrangement of the tentacles of *H. pilatus* with reference to the mesenteries was made in the series of transverse sections and also by an examination of the disk, and the results obtained were essentially the same as Carlgren's. I was not able, however, to distinguish any difference in the position of the tentacles over the endocoels bounded by the imperfect mesenteries, though on theoretical grounds it is probable that some difference does exist, and, furthermore, the study of sections seemed to indicate that the tentacles over the directive endocoels were situated a little nearer the mouth than were the others represented as being in the same cycle in Fig. 2; an examination of the disk failed, however, to confirm this appearance.

As regards the systematic position of *Halcurias*, a few remarks are in order. I at first (**'93**) assigned it to the family Halcampidae, but later (**'98**) deemed it advisable to separate it from that family and refer it to Hertwig's Antheomorphidae. Carlgren in the mean time had established for his *Endocoelactis* the family Endocoelactidae. There are apparently three courses open for the disposal of the genus. It may be referred to a family already existent, the definition of the family being changed, if necessary, to accommodate it; or it may be taken as the type of a distinct family, as Carlgren has done; or, finally, it may be separated altogether from the Hexactiniae and regarded as the type of a separate tribe.

It seems to me that this last procedure is quite unnecessary, and would probably be entirely out of harmony with the phylogenetic relationships of the genus. We have learned within recent years how extensively nearly allied forms may differ, and how great are the modifications which the hexactinian type may undergo. The entire facies of *Halcurias* is that of an hexactinian, and it may furthermore be pointed out that instances of the occasional endocoelous development of mesenteries have been already recorded by G. Y. and A. F. Dixon ('89) in *Bunodes thallia* and by Haddon ('98) in *Actinioides dixoniana* and *A. papuensis*.

If, then, the third possibility be excluded, *Halcurias* must either be assigned to an existent family, the endocoelous development of mesenteries being regarded as of minor importance, or this feature may be considered of sufficient importance to warrant the establishment of a separate family. I have already indicated my belief that the peculiar mode of development of the secondary and tertiary mesenteries is of minor importance and see no more reason for separating *Halcurias* as the type of a new family than I do for separating an octamerous sagartian, or one with a multiplicity of mouths and many siphonoglyphs, from the rest of the members of that family.

It remains then to consider what the forms may be with which *Halcurias* may be associated. As Carlgren has remarked, and as I have indicated by the position to which I have referred it in previous papers, *Halcurias* occupies a position near the base of the hexactinian stem. The small number of perfect mesenteries, the occurrence of reproductive organs on all of them, the absence of a distinct sphincter muscle, the simplicity of the margin, are features which, when combined in one individual, indicate for it a somewhat low position. Are there other forms which present a similar combination of peculiarities associated with the development of an adherent base?

Two forms suggest themselves in this connection, namely, *Gonactinia prolifera* and *Protanthea simplex;* but both of these present peculiarities which render their association with *Halcurias* inadvisable. They both have but eight perfect mesenteries, the remaining ones, eight in *Gonactinia* and about eighty-eight in *Protanthea*,[1] being imperfect, and the ciliated lobes are lacking in their mesenterial filaments. On account of these peculiarities it seems to me that these two forms must be grouped together in a family, Gonactiniidae, as Carlgren ('93) has proposed, and *Halcurias* cannot be placed with them. The family Gonactiniidae must, I believe, be placed among the Hexactiniae, as indeed must all the forms which I have included in the past in the order Protactiniae, as well as those which Carlgren has referred to the Protantheae. The discovery of hexactinian mesenteries in certain Edwardsiae, already referred to, necessitates either the abolition of both this order and that of the Protactiniae, or else an extension of the latter to include both the Edwardsiae and many of the Halcampidae, and it seems to me that the former step is the most practical and the most in accord with a correct phylogenetic scheme. Not that I mean by this that the stages of development shown by the members of the group do not represent phylogenetic stages in the evolution of the Hexactiniae. Certainly no one will imagine that what has so long been regarded as the Edwardsian type of structure is not in reality a primary phylogenetic condition, even though we are now obliged to regard the existing Edwardsiae as true hexactinians which secondarily in some cases may represent the more primitive condition.[2] The facts of embryology speak too strongly regarding the Edwardsian stage to allow of question as to its past occurrence, and I believe that there can be as little question regarding the stages which I have supposed to intervene between the Edwardsiae and the typical Hexactiniae, even though the forms which to-day represent these stages do so possibly only on account of secondary modifications.

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[1] *Protanthea* has four imperfect mesenteries which make pairs with the four lateral perfect mesenteries, and twelve others arranged in pairs in the primary exocoels, all being fertile and provided with mesenterial filaments. In addition to these there are, however, as in *Halcurias*, a number of short, narrow mesenteries confined to the upper part of the column and standing in relation to the tentacles, of which there are about ninety-six.

[2] Compare Van Beneden, Les Anthozoaires in Ergebnisse der in dem Atlantischen Ocean, etc., ausgeführten Plankton-Expedition der Humboldt-Stiftung. II. 1898.

It seems inadvisable then to associate *Halcurias* with *Gonactinia* and *Protanthea*, but there still remains a possible association with Hertwig's Antheomorphidae. Unfortunately the forms upon which this family was founded are insufficiently known, but it seems to me that there are reasons for maintaining the position I have already (**'98**) advocated, that the nearest allies of *Halcurias* at present known are to be found in the family Antheomorphidae. I find myself obliged, however, to recede from the position I held in 1898 as to the distinctness of this family and to return to my earlier opinion, which has received the approval of so critical a taxonomist as Haddon (**'98**), that the Antheomorphidae should be included in the family Actiniidae, and if this view be accepted it is necessary to refer the genus *Halcurias* to that family also.

This will necessitate no important modification of the definition of the Actiniidae given by Haddon (**'98**), but as I shall have occasion in the immediate future to consider the family in some detail, I shall postpone a discussion of its delimitation for the present.

UNIVERSITY OF MICHIGAN,
November 10, 1900.

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OBSERVATIONS
ON THE HABITS AND NATURAL HISTORY OF AMPHITHOE LONGIMANA SMITH.

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In the present paper I have given the results of my observations made at Woods Holl, Mass., during the past summer on a species of amphipod, *Amphithoe longimana* Smith. Comparatively little is known of the habits of amphipods, and most of what is known has been collected from scattered and casual observations. There is a value in getting together all the facts that can be obtained concerning any one species of animal, so that they may be viewed in their *ensemble* and thus give us some idea of the general life of the creature. For this reason it was deemed best to devote the short time that could be given to the study of amphipod behavior mainly to the observation of a single species.

Throughout the paper I have used many terms which imply the existence in the animal of certain psychical states, such as hunger, fear, and courage, without intending to affirm that such psychical states really exist in the animal's consciousness, or even that the animal possesses consciousness at all. It is difficult to describe the behavior of an animal without the use of terms which have certain psychological connotations. Such terms are here used simply as a matter of convenience in describing actions simply as actions. The Crustacea may or may not be "Reflexmachinen," and Bethe and others may or may not be right in denying that they possess consciousness ; but, however this may be, descriptions of actions in psychological terms stand for certain peculiarities of conduct that could not otherwise be easily described, and if the sense in which such terms are used is understood, no confusion need result.

Amphithoe longimana may be obtained in large numbers from the eel pond near the laboratory by simply drawing a net over the eel-grass. It is abundant during the summer months, a period when most of the other species of amphipods suffer a marked diminution in numbers. It is quite hardy, and may be kept alive for months in small glass dishes, if they are kept covered and the sea water kept fresh by a small piece of Ulva. Observations on this species were carried on for nearly three months. Specimens were kept isolated in small dishes and daily observations made and recorded. I was thus able to follow the histories of quite a number of individuals for a considerable period.

Specific Description.

Body slender. Eyes round. Lateral lobes of the head truncated in front. Antennules slender, about as long as the body, the second segment a little longer, but much more slender than the first; third segment from one-third to one-half the length of the second; flagellum much longer than the peduncle. Second antennae shorter than the first, but usually with a longer peduncle; last segment of the peduncle a little longer than the preceding one; flagellum shorter than the two preceding basal joints.

Second, third, and fourth epimera much longer vertically than wide; fifth epimeron about as long as the fourth, but broader and excavated at the upper posterior angle; lower margins of the epimera furnished with very short setae. Postero-lateral angles of the abdominal segments not acute.

First gnathopods in the male elongated, the first joint produced into a rounded lobe at the antero-distal angle; carpus narrow, nearly as long as the hand, and thickly setose on the posterior margin; hand very long and narrow, slightly incurved, and of nearly the same width throughout, although slightly widened near the base; lower margin setose; palm very short, transverse, and rounded at the outer angle; dactyl very large, dentate, and projecting far beyond the palm when closed. Second gnathopods much stouter than the first; first joint with a rounded lobe at the antero-distal angle; hand much broader and stouter than in the first pair; palm oblique, with a deep sinus near the strongly produced outer angle; dactyl scarcely projecting beyond the palm.

In the female the gnathopods are much shorter and weaker than in the male; the hand in the first pair is less elongated, and the palm is more oblique and more broadly rounded at the outer angle. In the second pair the sinus in the palm is not so deep, and the outer angle not so prominent as in the male.

Peduncle of the first pair of pereopods rather slender, much longer than the rami, and reaching nearly to the tip of the peduncle of the second pair; inner ramus of the second pair of uropods about as long as the peduncle. Posterior pair of uropods with the rami scarcely half as long as the peduncle; rami subequal in length, the broader, more or less oval inner one with a short spine at the inner posterior angle and several setae on the transverse distal margin; outer ramus with the usual stout hooks. Length 6–9 mm.

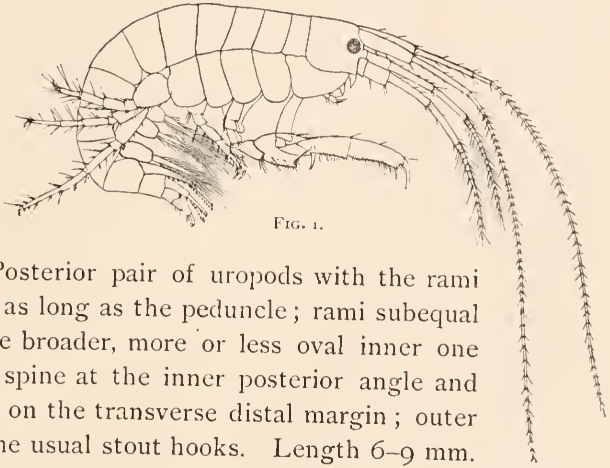

Fig. 1.

In the older specimens the antennae are relatively more elongated, and the hands of the male relatively longer and narrower. The eyes in the living specimens are red, but become black in specimens preserved in alcohol.

Habitat.

The range of this species as reported by Professor Smith is from Vineyard Sound to New Jersey, and it has been reported from Provincetown, Mass., by Richard Rathbun. It is not uncommon among the seaweed near the shore, and it has been taken at the surface in the vicinity of Woods Holl in the tow net. Its occurrence at the surface is probably due to its having been carried away from the shore by tide currents, as it has a strong tendency to keep among objects of shelter.

Its most favored habitat seems to be the eel-grass, where it finds a convenient substratum upon which to construct its nests. This species is much more common in the eel pond at Woods Holl than outside; the abundance of eel-grass and various algae and the quiet water being conditions which doubtless favor its perpetuation. It is not found on the muddy bottom and does not occur abundantly in the seaweeds near the bottom, but it may be obtained in quantity from the masses of eel-grass at the surface.

Enemies.

In common with most amphipods, Amphithoe is doubtless preyed upon by fishes, and it certainly affords one of the principal articles of food of the small but voracious jelly-fish Gonionemus. The latter form, however, owing to its unfortunate attractiveness to the zoölogists frequenting Woods Holl, is in danger of not continuing to be a very destructive enemy. It is very common to find Gonionemus with Amphithoe in its stomach. This crustacean falls an easy victim to its enemy, as it often makes surprisingly little effort to escape, owing possibly to a narcotizing effect of the poison of the nettling organs of its captor. I have seen an Amphithoe while swimming vigorously strike against the tentacles of the jelly-fish, suddenly stop, and remain almost perfectly quiet while it was being engulfed.

Food.

Amphithoe lives chiefly upon seaweed. The alimentary canal may usually be seen to contain numerous fragments of red or green algae. Pieces of Ulva that are kept in dishes containing these amphipods soon exhibit gnawed margins and, after some time, a marked diminution in size. I have often observed the process of feeding. The Ulva is gnawed directly by the mouth parts, without being previously torn away by the gnathopods. The quantity of algae eaten, judging by the amount of excrement voided, is very considerable. In order to ascertain how rapidly the excrement accumulated, a specimen with an abundance of Ulva was placed in a clean dish, and it was found that one hundred and forty-six masses accumulated in twenty-four hours. These masses consisted almost entirely of broken-up cells of Ulva, the contents of which had been digested out. By making a very rough estimate based on the size of these masses, it was calculated that the amount of food eaten by the animal in twenty-four hours was about equal to one-tenth of its bulk.

Amphithoe is by no means a strict vegetarian, for it will devour animal food with great eagerness when it can be obtained. It is very fond of bits of flesh of almost any animal, not excluding that of its own species. When aware of the presence of food sufficiently near its nest to be seized without letting go its hold, it will dart out with a quick movement, grab the food with its gnathopods, and suddenly retract itself inside its domicile. When the food is brought in, it is held by the gnathopods while being devoured.

Movements.

Of the movements performed by Amphithoe, the beating of the pleopods is the most constant and uniform. Whether the animal is swimming, crawling, or lying quiet, the pleopods are continually engaged in their regular to and fro movement. The motion of these appendages while the animal is at rest serves to create a current of water past the gills in front, and thus aids in respiration. The abdomen, except during swimming, is held strongly flexed, forming a sinus, at the posterior end of which the bases of the pleopods are attached, the tips pointing forward. Small particles suspended in the water may be seen to be drawn in at the sides of this sinus and thrown out at the anterior end, thus indicating the course of the current.

The rhythm of the motion of the pleopods, like the respiratory movements of the higher vertebrates, goes on in a regular way as a rule, but may be checked by impulses from the higher nervous centers. When the animal changes its position, or executes any other decided movement, the pleopods may cease their action for a moment, but soon resume their regular beat as before. Commonly during swimming the pleopods beat more rapidly, but this is not always the case. When the swimming ceases they drop back into their usual rhythm, whether faster or slower than before. In their motion the three pairs of pleopods act as a unit, keeping perfect time like well-trained oarsmen. If the abdomen be removed from the rest of the body, the pleopods, after a few spasmodic movements due to the shock of the operation, continue to beat rhythmically for several minutes; the three pairs all move with the same rhythm, though somewhat more slowly than before the operation. Moreover, if a single segment with its pair of appendages be isolated, the rhythmic motion of the appendages still goes on for some minutes, but gradually becomes slower and more irregular, until nothing but small twitches indicate the existence of irritability.

When the animal is in a vigorous condition the beat of the pleopods is rapid, but when the creature becomes weakened the beat becomes slower, yet as long as life lasts the pleopods continue their movements. The beat of the pleopods may still persist after the rest of the animal refuses to respond to any sort of stimulation.

The swimming of Amphithoe is mainly effected by the pleopods. The first impulse, however, is gained by the sudden extension of the abdomen, which gives the body a rapid forward movement. The abdomen is then held in an extended position and the pleopods, which then hang nearly at right angles to the body, serve to continue the forward motion. When swimming against the force of gravity the motion of the pleopods alone is not sufficient to keep the body going, and when the original impetus becomes exhausted the abdomen is bent forward and again suddenly extended, giving the animal a fresh start. The flexure of the abdomen before every stroke tends to draw the body backward. This, combined with the weight of the animal, causes ground to be lost between every stroke. Swimming towards the surface is therefore accomplished by a series of springs, between each of which the animal falls back more or less. While swimming horizontally the beating of the pleopods is all that is required to keep up the motion; specimens may be seen swimming about for a considerable time without employing the abdomen.

Amphithoe has a decided disinclination for continuous swimming. Ordinarily it makes only short excursions from one place of concealment to another and generally stops upon meeting with the first solid object that comes in its way, although when situated where there is nothing to which it can lay hold it may swim for some time in a uniform manner. It may swim in various ways, on its side, or with either the dorsal or the ventral surface uppermost, and apparently gets along with about equal facility in any of these positions.

The beat of the pleopods tends to propel the body not in a straight line forward but in a circular course. The pleopods being on the ventral side tend to cause the body to veer around towards the dorsal side. When the body is held somewhat concave on the ventral side, as it often is, this tendency may be balanced or overcome by the tendency to move in circles in the opposite direction. Such a condition is analogous to a person rowing on one side of a boat with the rudder turned toward the side of the oar. By having the body extended to the right degree a straight course may be maintained. The direction of movement is often changed by the animal turning now on one side and now on the other. Circular movements in one direction are thus compensated for by circular movements in another as the animal turns over and a certain general direction of motion is maintained. When swimming on the back a nearly straight course is kept by rolling the body slightly to the one or the other side. Rolling is probably effected by the movements of the hinder pairs of thoracic legs. When the animal is swimming these legs project outward and backward. A downward stroke of these appendages on one side would push the same side of the body upward and roll it over toward the opposite side. In a larger species of amphipod, whose movements are not so exceedingly rapid as those of Amphithoe, I was able to see that the rolling of the body was effected in just this way. It is highly improbable that in Amphithoe a different method would be employed to produce the same result. However this may be, it is certain that

Amphithoe steers itself while swimming by altering the extension of the abdomen and by rolling from side to side. Lateral bendings of the body could not be seen to play a part in directing the swimming motions, although I have observed this method of steering employed by other amphipods.

Amphithoe longimana, like many other amphipods, is unable to walk over a plane surface. When out of water it is able to make indifferent progress by the characteristically amphipodan gliding movements produced by alternately flexing and extending the abdomen. It is utterly incapable of leaping like the sand fleas and some of their aquatic relatives. Owing to its compressed form, it is unable to maintain itself upright while out of water, or even in water, unless it has some object to which it can lay hold. In its characteristic habitat among the seaweed, Amphithoe crawls with considerable agility. The principal organs for crawling are the second antennae, the two pairs of gnathopods, the third and fourth pairs of pereopods, and to a certain extent the abdomen. The antennae are thrown over objects and flexed, thus tending to pull the body upward and forward. The gnathopods are used to seize objects in order to pull the body along. The two following pairs of appendages are employed much as the walking legs of insects, although they move in a nearly vertical plane. The abdomen assists in locomotion by being thrust forward beneath the body until the tip is hooked on to some irregularity of the surface over which the animal is moving when it is extended, thus giving the body a forward impulse. The movement recalls the leaping motion effected by the abdomen in the sand fleas. In fact, very similar motions are performed in both cases, but in Amphithoe the motions are much less rapid and energetic. The ambulatory movements of this species are never rapid, owing doubtless to the necessity for keeping the body from falling over on its side. The last three pairs of thoracic legs, although not used directly for locomotion, are indirectly of service in holding the body upright. These appendages, which are bent over the back and have their claws pointing forward, are used to hook on to objects and thus act as organs of support while progression is effected by the other appendages of the body.

Ordinarily Amphithoe lies in its nest, with the antennae protruding from the opening at the end. The lower pair of antennae are generally held motionless. The upper pair, however, are usually seen to be moving to and fro, sweeping about in almost every direction. Occasionally their motion is suddenly checked and they are held motionless for a time, but soon their movement is resumed. The significance of these movements will be discussed in a later section. The two pairs of gnathopods are used for a variety of purposes. Occasionally the antennae are bent downward and seized by the gnathopods and the flagella drawn through the space between the dactyl and the palm. The function of this act is probably to strip off any foreign bodies that may become attached to the antennae. The gnathopods are frequently employed to grab passing objects and to reach out and pull in bits of seaweed for the construction of the nest. They are used also for holding the food that the animal is eating and for carrying bits of food to the mouth, where they are taken by the maxillipeds. While not exercising any of their normal functions they may often be seen going through the motions of grasping, with nothing to grasp. This restless activity of the gnathopods seems to be nothing but the exercise of the grasping reflex called forth by some unknown stimulus and having no useful result. The act is performed in all degrees of completeness, from a definite grasping motion to a mere nervous twitch. The mouth parts perform many motions when the animal is not masticating food. These movements, which resemble the normal motions of mastication so far as could be observed, apparently have no functional significance. They take place in specimens kept for a considerable time in dishes in which there is nothing that could serve for food. Like the grasping actions, they are movements which are called forth without the normal exciting cause.

One of the most curious actions which Amphithoe performs is its reversal of position in the nest. If the antennae be somewhat roughly struck with a needle, or even if a threatening object suddenly appears close in front, the head and antennae will appear at the other end of the nest. As the nest is a tube but little wider than the body of its occupant, no one who watches the operation can fail to have a feeling of admiration for the neatness and extreme quickness of this acrobatic feat. The animal executes this "about face" with such rapidity that it is only after watching the operation repeatedly that one can determine how it is effected. As the animal lies in its nest the abdomen is bent forward and the posterior pairs of thoracic legs are directed backward, their claws being usually hooked into the walls of the nest. When about to turn around, the abdomen is thrust forward, its terminal hooks caught in the nest; the tip of the abdomen, therefore, forms a fixed point, and the insertion of the thoracic legs forms another. The contraction of the legs would therefore pull the middle and anterior parts of the body backward. When the head is pulled back some distance, extension of the body occurs, forcing the anterior part of the body through to the other end of the nest. The head end being reversed, the abdomen is loosened and quickly flexed again under the body. The whole operation is completed in less than a second, and the animal may be made to repeat the performance several times in rapid succession.

Nests and Nest-Building.

The nests of Amphithoe are tubular structures which generally exceed somewhat the length of the animal. They are made of a web-like material which, under the microscope, may be seen to be a network of exceedingly fine threads. The nests are usually constructed among the branches of the red seaweeds or upon the leaves of eel-grass or the fronds of Ulva. When built upon Ulva the nest is generally located in a wrinkle or fold of the surface which affords a partial shelter. The nest is open at both ends and is of about the same diameter throughout. Foreign materials, such as bits of seaweed, are usually woven into the nest and greatly add to its efficiency as a means of concealment. Amphithoe frequently leaves its nest, but I could find no evidence that it would return to its own nest more readily than to any other; it will simply enter the first unoccupied nest that comes in its way. When established in a nest Amphithoe is driven out only with difficulty. A member of its own species that approaches is grabbed at and usually driven off, and the creature appears to be on the alert to keep out all intruders. The approach of a more formidable-looking object causes the animal to retreat farther back into its nest. If the antennae are stroked with a needle, a sudden somersault will be executed and the head will appear at the other end of the nest. Then it usually requires quite a series of pokes to make the creature quit the nest entirely. The instinct to remain in the nest when danger threatens presents a marked contrast to the quickness with which flight is made when the animal is roaming free.

A new nest is constructed in a remarkably short time, often in less than a half hour. If a few specimens be placed in a dish of sea water containing a little seaweed, nests will be woven on the seaweed and on the lower surface of the dish, and in a short time the number of nests may greatly exceed the number of specimens. Those localities are chosen which give the animal a maximum of contact with solid objects. In dishes in which specimens were kept I have nearly always found several nests along the angle between the bottom and sides, although the seaweed kept in the dishes afforded localities better adapted for concealment. The choice of a spot for a nest is apparently largely a matter of thigmotaxis. When the animal remains in a spot for some time, the nest-building activities begin, and where contact with different sides of the body is secured, as between the branches of seaweed, in the wrinkle of an Ulva frond, or in the angles of a glass dish, it remains quiet. If Amphithoe is observed while constructing its nest, the first and second pairs of pereopods will be seen to be busily engaged in moving back and forth from point to point along the surface on which the web is being laid down. The first and second pereopods contain large glands which are connected with a duct which opens at the tip of the claw. The material for the web is secreted by these glands and probably hardens soon after its emergence, like the web of a spider. A very fine thread of web may frequently be seen passing out from the small opening at the tip of the claw. As the tip of the claw touches one point after another, the web, as it is drawn out, is fastened to different places. By moving back and forth and rolling around during the weaving process, the animal constructs its tubular dwelling. Several specimens from which I clipped the claws from the first two pairs of pereopods were kept for several days and did not construct a single nest.

During the construction of the nest, Amphithoe will reach out and draw in bits of algae and other objects that lie near and incorporate them into its dwelling. In a few cases I have seen long pieces of algae bitten in two and used for this purpose. As Amphithoe lives largely on algae, this biting may not have had any special reference to nest-building, but may have been a manifestation of the ordinary reaction to food. In Microdeutopus, Smith has observed that the excrement of the animal is worked into the web; but in Amphithoe, whose nest-building habits seem to be very similar, no such process could be observed. The excrement is passed out of the nest, accumulations of it usually being observable near the two ends.

Moulting.

Amphithoe was found to shed its skin more often than was anticipated. Most of the specimens I kept isolated as long as a week moulted once, and out of four specimens in which I have records of the dates of two successive moults of the same individual the interval between moults in three cases was seven days, and in the other case eight days. These specimens were of the usual size. How rapidly moults occur in different periods of the life history of this species I cannot say.

The process of moulting in Amphithoe occurs in the same manner as has been described in other species of amphipods. The skin splits transversely along the line joining the head and thorax, and on either side of the thorax is a longitudinal split which occurs between the upper margins of the epimera and the lower margins of the thoracic rings. This split may extend along all the thoracic segments. The head and antennae are pulled backward out of their investment and the posterior part of the body is pulled out forwards, the old skin, after being shed, remaining intact except at the lines just mentioned. The moulting process takes several minutes at least and is accompanied by considerable muscular effort to get out of the old skin.

In the several cases in which I observed the process, Amphithoe leaves its nest to divest itself of its skin, and I have never observed a moult in a nest but always some distance away. After moulting the animal is rather quiet and cannot easily be enticed from its nest by food. I have observed several cases in which death occurred during the moulting process. In one case moulting was not completed for several days. The specimen was observed August 17 with the head and tail ends drawn partly out of the old case. The next day the head and antennae were still not completely drawn out, but the rest of the skin was kicked off. On August 21 it was still in the same condition, the feeble beating of the pleopods giving evidence of failing strength. On the next day it died, the head and antennae still only partly extricated from their old covering. In several cases the antennae were observed to become broken off in the process of moulting, but I have seen no cases in which other appendages became lost in this way. The antennae are the appendages most liable to more or less complete loss from other causes, but owing to the rapidity with which these organs can regenerate this loss can produce only a temporary inconvenience. The cast-off skins are found sometimes on the bottom, and often floating on the surface of the water, and in a short time after they are shed become filled with swarms of protozoa.

The Seat of Smell.

Much has been written concerning the seat of the olfactory sense in the Crustacea, but most opinions on the subject have been based on morphological instead of experimental evidence. The work of May and Bethe affords good evidence that in the decapod Crustacea the seat of the olfactory sense, or, as Bethe prefers to call it, of chemoreception, is in the first antennae, as analogy with the insects would lead one to suspect. The first antennae are not, however, according to Bethe, the only seat of chemoreception. In Carcinus the removal of the first antennae as far as the first basal segment is followed by a marked diminution of the power of reaction to chemical substances in the water. A Carcinus when the eyes are blackened over will find pieces of food when placed at some distance, by the sense of smell. When the first antennae are removed at the first basal segment, Bethe found that food may be placed as near as 10 cm. to the animal without calling forth any efforts to obtain it. When the food is brought close to the mouth or close behind the animal without contact with the body, it is seized and eaten. The first antennae, therefore, while they may be the main, are not the sole source of the reception of olfactory stimuli.

My own observations on Amphithoe led me, before becoming acquainted with Bethe's results, to infer a double seat of the sense of smell. In Amphithoe, as in Carcinus, the first antennae seem to be the most important olfactory organs. While the animal is at rest in its nest the antennules are kept swaying to and fro in different directions, as if they were being employed to explore the surroundings. If a small bit of flesh is held on a needle or in a fine pair of pinchers and carefully brought near the animal, the antennae check their random movements and make one or more strokes in the direction of the bit of flesh; often the antennae are held for some time in the direction of the object. On bringing the flesh nearer, the animal may be seen to adjust itself in the nest for a sudden spring, and if the flesh is sufficiently near to be touched by the antennules the amphipod makes a sudden dart from the nest, seizes the object, and draws quickly back again, never letting go its hold, however, of the nest. The animal as a rule readily distinguishes between the contact of flesh and that of a body not serviceable for food. Only rarely does touching the antennule with a needle call the animal forth from the nest. It may be deceived more often if, when excited by the presence of meat near by, one of its antennules be touched with a needle; then it may dart out towards the needle and even seize it. But the animal responds much more surely, as I have found by repeated experiments, when the antennules come in contact with the food itself, even when the animal is excited by the presence of food in its vicinity. The darting forth, therefore, is apparently caused, not merely by a tactile stimulation, but by a chemical stimulus from the food. The antennae are delicate tactile organs, and tactile stimuli may assist in calling forth the actions which result in the seizing of food, but tactile stimulation alone generally fails to accomplish this result.

After Amphithoe has made a meal of fleshy diet it becomes quite indifferent to the presence of that kind of food in its vicinity and no longer darts forth to grab bits of flesh brought in contact with its antennules. Different individuals present very different degrees of eagerness for animal food, owing doubtless to varying intervals of time since their last repast.

Sight has probably little to do with the food reactions of the animal. When the head is completely withdrawn in the nest the animals often give signs of perceiving food and dart after it when brought in contact with the antennules. In many cases the nest is so opaque that the animal cannot see through it with any distinctness, and under these circumstances, when the head was entirely withdrawn into the nest, I have often brought bits of meat so they would be touched by the antennules only when they were strongly bent backwards. Although the meat was out of sight, the amphipod would dart out, bend backwards, and seize the morsel. If the desired object is out of reach of the antennules, the amphipod will not spring for it, although it may be seen to make ready to do so. It will not go to the length of leaving the nest to seize food, even if its conduct betrays evidence of keen hunger. An object near enough to be struck by the swaying of the antennules is sufficiently near to be seized by the animal without letting go its hold of the nest. It is a noticeable feature of the species of Amphithoe and related genera that the antennules are, roughly speaking, of about the length of the body. This feature is not improbably correlated with the similar tube-dwelling habits of these forms, the length of the antennules gauging the length of a safe and successful spring.

The effect of removing the antennules of Amphithoe is greatly to lessen responsiveness to olfactory stimuli. The shock of the operation has a very temporary effect, for in a few hours the animals behave with their usual activity. Meat brought in contact with the second antennae is generally not seized. This, however, is not always the case, for in several instances I have found that contact with the second antennae causes the grasping reflex. I was inclined at first to attribute a certain olfactory sensibility to the second antennae, but I found later that the animal reacts about as well to olfactory stimuli when both antennae are removed as when the second alone remain. In specimens with both antennae removed near the base, leaving only the first joint of the peduncle of each pair, there was no reaction to food placed in what would have been within easy reach of the antennae before their removal. If a piece of meat is placed about 2 mm. from the mouth of the amphipod, it is generally allowed to remain untouched for several seconds and then suddenly seized and eaten. The morsel is seized by the gnathopods and at the same time bitten at with the mouth parts. The reaction is not an immediate one, such as is brought about by contact of the antennules with food. It appears to be necessary for the food to remain awhile in close proximity to the animal before its edible nature is perceived; when this occurs the seizing takes place quickly enough. I have tried to induce the animals to take bits of substance of the general appearance of fragments of meat and brought very close to the mouth parts, but they are apparently able to distinguish, before any contact with the object occurs, whether or not it is of an edible nature. It is true that Amphithoe often grasps objects that lie near by, pulls them back, and incorporates them into the structure of its nest, and it might be inferred that the seizing of meat lying close to its mouth by a specimen with both antennae removed is an expression of the nest-building instinct to seize any small object within reach for building material. The reactions in the two cases, which I have observed many times, differ. An object used for the construction of the nest is reached for and pulled back to the nest and not as a rule brought in contact with the mouth. A bit of meat is grabbed at and bitten at in the same act. This difference in reaction and the fact that the animals seize bits of meat when they cannot be induced to pay attention to other objects of similar appearance convinced me that the reaction to food was caused by chemical stimulation from diffusing substances in the water.

Removal of the second pair of antennae, the first being left intact, was not found to exert any marked influence upon reactions to chemical stimuli. The second antennae may transmit olfactory stimuli; it would be difficult to prove they do not in a certain degree, but the evidence obtained does not justify us in attributing to them this function. When, after removal of the first antennae, Amphithoe responds when food is brought in contact with the second antennae, the reaction may be due to the animal becoming aware of the presence of food through some other organ, contact with the antennae indicating that the food is sufficiently near to be seized. In other words, the reaction may be due to purely tactile stimulation, the animal being keyed to this reaction by the excitement of olfactory stimuli from some other organ. What other organ, or organs, may serve to transmit olfactory stimuli is uncertain. This has not been determined in the decapod Crustacea, which afford the only other instance in which a double seat of the olfactory sense has been suspected, or in fact in which, so far as I am aware, any experimental evidence has been adduced as to any location of this function at all. It seems probable that some of the mouth parts have some olfactory function, as they afford the most obviously appropriate location for such a sense. Owing to its small size, Amphithoe is not a favorable form in which to decide this question, and the attempt to do so was not made.

Color and Color Changes.

One cannot but be struck, when examining a number of specimens of this species, with the marked differences in color presented by different individuals. Some are bright green, like the bright green seaweeds; others may be nearly colorless; a few are of a light blue green tint, and many range from a light to a dark reddish-brown. The same individual may take on, at different times, all these varieties of coloration. The color differences are produced by the variation of five elements: (1), the color of the chitinous integument; (2), the color of the blood and tissues; (3), the contents of the alimentary canal; (4), the color of the sex glands; (5), the pigment cells. The first of these factors is, perhaps, the least important and is not subject to great variation. The exoskeleton over most of the surface of the body is colorless; on the antennae it is marked with transverse reddish-brown bands which give the light reddish-brown annulations of these organs. This color is seen as distinctly in the shed skin as in the living animal.

The color of the tissues and blood is subject to great variation. The green color of Amphithoe, or the blue green tint when it occurs, is due to some coloring matter that is uniformly diffused throughout the animal. In some specimens there is a sufficient amount present to give the animal a brilliant emerald green, but many may be found in which not the slightest trace of green coloration could be detected. This green color may be seen to undergo marked changes in intensity if individuals be watched for several days. The blue color is much rarer. One specimen in which this blue coloration was strongly marked was kept under observation for several days. After five days most of the blue color had disappeared, the green becoming more nearly like the typical green of other forms. After six days the green was not to be distinguished from the ordinary type; the green color then gradually became fainter, and on the ninth day the tissues were whitish, scarcely a trace of green being visible. During all this time the specimen ate abundantly of green algae, judging from the amount of excrement consisting of Ulva cells that accumulated in the dish.

The contents of the alimentary canal influence to a considerable extent the general color effect produced by the animal when seen by the naked eye. If they consist largely of green Ulva, they tend to give the animal a greenish appearance. If the Ulva has been subjected some time to the action of the digestive juices and become a yellowish color, it tends to give the animal a corresponding yellowish aspect. Light becoming colored by passing through the alimentary canal is reflected and re-reflected in the tissues and tends to make them appear a corresponding color. I have several times cut off parts of the body to see if their color might not have been due to this cause; but whatever effect this factor may have it is certain that the green color of the blood and tissues is not entirely caused in this way as it may easily be observed in the isolated appendages. The part played by the sexual glands in the coloration of this species varies greatly owing to the variation in the size of these organs.

It is to the pigment cells that the most marked changes of color are due. These cells are of two kinds, — reddish-brown pigment cells, and cells with a pale green pigment. The latter play an insignificant part in the coloration of the animal, as they are pale in color and few in number, there being often not more than a dozen on the entire surface of the body. The pale green color appears most clearly in transmitted light; in reflected light they are of a silvery hue. Their size is about the same as the largest cells with red pigment. Like the latter, they are very richly branched, but were not seen to undergo much variation in the distribution of their pigment. They are mainly confined to the epimera, being usually situated near the lower margin.

The most important elements in determining the color changes are the reddish-brown pigment spots. These spots are scattered all over the body and are found also on most of the appendages, especially towards the proximal end. When extended the pigment spots are large and very richly branched, forming most beautiful objects when seen under the microscope. When fully contracted these spots assume the form of round dots, and all stages of expansion may be seen in different specimens, or even in the same specimen, between the most contracted and the most expanded state. There are a few large spots near the lower edges of the epimera that are generally found in an expanded condition. Even when most of the spots over the surface of the body are contracted, these few spots, which may be not more than a dozen on each side, are usually conspicuously large. This circumstance affords a convenient means of distinguishing *Amphithoe longimana* at a glance from other species of the same genus.

The pigment spots of Amphithoe apparently contain but one kind of pigment. The whole system of chromatophores is much less complicated than that which Gamble and Keeble[1] found in *Hippolyte varians*, and the power of sympathetic color changes in relation to surrounding objects much less perfectly developed. Amphithoe may be said to adapt its color to its environment, but in only a rather rough way compared with the remarkable protective color changes of Hippolyte. Specimens taken from the eel-grass are very apt to be of a greenish color, and specimens taken from among masses of red seaweed are usually colored somewhat like their environment. While on the eel-grass, they are usually exposed to the light and the pigment spots are in a contracted condition. This allows the green color of the tissues to be seen, and the animal has, consequently, a greenish aspect. In the masses of red seaweed the animal is usually more shaded and the pigment spots become expanded, giving the animal a reddish tint which helps to conceal it in its environment. Moreover the alimentary canal in specimens found in the red seaweed often contains a greater or less quantity of this alga, and this also helps to color the animal in a protective manner. So far as the green color of the tissues is concerned there appears to be no difference between the animals from different habitats. The change from green to reddish, or the reverse, completes the range of adaptive color variations in this species so far as they are induced by changes in the environment. And this kind of color change is the one best adapted to afford protection to the species in its usual habitat.

The pigment spots of Amphithoe change very slowly; it generally requires some hours to effect a change from the expanded to the contracted condition. It was found by several experiments that exposure to bright light causes the pigment spots to contract, while specimens that have been kept in the dark for several hours generally have the pigment spots much expanded.

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[1] *Quart. Journ. Micr. Sci.*, 1900.

Sexual Habits.

Concerning the sexual habits of Amphithoe I have little to add beyond what is known among other amphipods. The male carries the female about for a considerable period and maintains his hold against efforts to dislodge him with great pertinacity. The instinct to retain hold of the female is sufficient to overcome all fear, and it is difficult to separate the male without injury. The posterior part of the body may be cut off, and yet the anterior portion retains its hold of the female as long as sufficient vitality remains. Ordinarily the male retains his hold of the female by hooking the claws of his pereopods beneath the edges of the epimera. The gnathopods are not generally employed for this purpose, but are called into use when a sudden disturbance renders the hold of the male insecure. The female remains remarkably passive when carried about by the male. Her body is usually held quite strongly flexed and the male does the swimming for both, the female being transported as so much dead weight. While carried by the male the female seems much less responsive to stimuli than when free. When poked by a needle she often makes little motion, but the more alert male is generally aware of the disturbance and carries her away from the seat of annoyance.

The Disposal of Excrement.

In its natural position Amphithoe lies so that the excrement that is voided would be deposited in the nest. Yet the excrement is never found in the nest but at some distance from either end. At first I supposed that it was carried out by the current of water produced by the movement of the pleopods, but, after watching the animal, I noticed that when excrement was extruded the abdomen was bent forward and the gnathopods reached back and seized the mass as it was ejected from the intestine, and passed it out of the front end of the nest. This act was observed three or four times, but whether it is always performed when excrement is extruded I cannot state. In one individual lying outside a nest on the bottom of a glass dish I noticed that when excrement was passed the abdomen was bent forward and the mass seized by the gnathopods and passed forward, just as it is when the animal is in the nest. There was of course no use in seizing the excrement under the circumstances, but the act was performed in the usual instinctive way nevertheless.

Timidity and Pugnacity.

Specimens of Amphithoe are very ready to attack other amphipods that come near and drive them away. The animals appear to be on the alert to prevent any other individual from gaining access to the nest, — so much so that they very frequently spring out at a passing amphipod and bite at it in what appears to be a particularly vicious and hateful manner. The individual attacked does not, so far as I have observed, attempt any defense but precipitately flees from the spot. Anything that properly could be called a fight is never engaged in; a passing nip with the gnathopods, or bite with the jaws, is all that seems to occur in the nature of hostilities.

While ready to dispute the entrance of another amphipod from in front, Amphithoe generally quickly flees from its nest when an intruder enters from behind. One often sees the occupants of nests routed out by others entering in this way, and I have seen one individual that was expelled by another entering behind it swim around, enter the other end of the nest, and drive out the intruder. Courage in Amphithoe depends in great measure on whether the attack is made from in front or behind.

Outside the nest Amphithoe is very timid. It does not attack its fellows except by giving an occasional nip when accidentally colliding with them, and it flees quickly when disturbed. It can very rarely be induced to seize meat, however hungry it may be, and however carefully the food be presented. Even when in the nest, care has to be taken in offering the animal food lest it be alarmed by one's movements. This alarm is manifested by withdrawing a short distance in the nest. When an animal to which meat is presented withdraws in this way, I find that it is useless to attempt to induce it to take food for several minutes, until its fright wears away. Experience in feeding these animals soon enables one to tell whether or not they have become frightened by your actions.

So far as my observations go they indicate that Amphithoe has very little true pugnacity. It does not engage in a conflict in order to overcome an adversary, as many decapod Crustaceans do; it fights only in self-defense. The attacks on other amphipods passing by the nest are simply measures to keep out unwelcome visitors. Had not these forms the instinct to keep the nest to themselves, several individuals would often crowd into the same nest much to their mutual inconvenience.

Phototaxis.

Amphithoe, like most of the aquatic Gammaridea, is negatively phototactic. The specimens experimented with were placed in an elongated, rectangular dish contained in a box open at one end and above and blackened on the inside. When placed near a window, either in direct sunlight or so that rays of diffuse daylight fell obliquely into the dish, the animals would swim towards the end of the dish farthest from the source of light. When the dish was turned about, they swam back again to the other end. In lamplight they may be driven alternately from one end to the other by moving the lamp back and forth to opposite ends of the dish. The endeavor was made to make Amphithoe positively phototactic by altering the temperature and concentration of the sea water, but only negative results were obtained. The animals remain negative until the water is heated to about 90° Fahr., when little responsiveness to light remains. Further heating causes heat rigor to supervene. Increase of temperature may be carried to the point of producing death without changing the direction of the phototactic movements. It is not probable that cooling the water below the normal temperature would alter the phototactic response. Decrease of temperature, other things equal, tends to give rise to negative phototaxis in Orchestia, and increase of temperature has the effect of making this form more positive.

Raising the temperature has the effect of making *Gammarus mucronatus* positively phototactic, but an increase of only a few degrees beyond the point where its phototaxis changes produces heat rigor. Possibly Amphithoe might be rendered positively phototactic could it endure a somewhat higher temperature.

Neither increasing nor decreasing the concentration of the sea water was found to effect a change in the direction of phototaxis. A more extended account of phototaxis in the Amphipoda will appear in another paper.

Thigmotaxis.

The tendency of Amphithoe to keep in contact with solid objects is one of the most conspicuous features of its behavior. This tendency is apparently one of the fundamental instincts of the group, as it is exhibited very strongly by most of the Amphipoda. When placed among branched seaweeds, Amphithoe stops only after it works its way among the branches where there is contact on several sides of the body. In Ulva it comes to rest in a fold of the frond. When placed in a glass dish containing nothing but sea water, it swims about restlessly and eventually comes to lie quiet in the angle between the bottom and side. The instinct to crawl into an empty nest is an expression of the same tendency. Were it not for its thigmotaxis, the whole conduct of the animal would be very different from what it is. Many other instincts, like the nest-building instinct and the instincts associated with it, are built upon this fundamental reaction as a foundation. It does not seem improbable that the instinct of the female to remain perfectly quiet while carried about by the male, and even the strong propensity of the male to seize and retain hold of the female, may be but modified and specialized forms of thigmotaxis. Given variations of responsiveness to contact in different parts of the body, and variations in the manner in which the responsiveness is exhibited, we would have the means by which thigmotaxis might be modified by natural selection into more specialized forms of behavior. If the origin of the various forms of amphipod behavior could be traced, it would be found, I believe, that thigmotaxis is the mother of many instincts.

The Instincts of the Young.

When the young of Amphithoe quit the maternal brood pouch, they have a quite different appearance from the adult. They are whitish in color, with only a very few pigment spots, located for the most part on the epimera, each epimeron containing often but one spot. These pigment cells are of a greenish-gray color and apparently have none of the reddish-brown pigment which is found in those of the adult. By reflected light they have a light greenish-silvery appearance much like the large light green cells of the adult, but they are very much smaller and much less branched. The head is relatively large, but the eyes are small and red and composed of only six ocelli, one of which is in the center surrounded by five others. The number of ocelli, therefore, increases very greatly as the animal grows older. Both pairs of antennae are short, the flagella of the first pair consisting of four joints and those of the second pair of but three.

The young when first hatched are in a feeble condition and are carried about for a few days in the brood chamber of the mother. Before they are hatched one can easily see the beating of the heart and the peristaltic movements of the intestine. Shortly before their emergence the young may be observed flexing and extending the body in the effort to break the shell. In one case I removed a lot of eggs from the brood pouch of the mother while they were hatching. Those that had not yet emerged were very vigorous in their movements within the shell. When hatched they were unable to swim, and their movements were irregular and little coördinated. The next day a few had died; the others could swim feebly, but none well, and if they became caught in the film at the surface of the water they were unable to overcome the surface tension and get free. In another case I removed from the brood pouch of a female several young that had been hatched only for a short time, as they were feeble and scarcely able to swim.

The swimmerets, however, beat rapidly, and the maxillipeds and gnathopods were in constant motion. The antennules were moved more than the antennae, but their movements were more jerky and irregular than in the adult. The next day several others came out of the brood pouch of the mother of their own accord. They were a little more active than those that had been removed the day before and exhibited apparently a faint negative phototaxis. They had been out only a short time when they began constructing nests. These nests were the same in shape as those formed by the adult, and the behavior of the young in relation to the nest was almost exactly like that of the older individuals. One of these young impaled on a needle was presented to one of the same brood lying in its nest. At first the animal gave signs of timidity and withdrew further into its nest. After some waiting the animal emerged a little and began waving its antennules in the usual manner. When they touched the food the creature darted out quickly, seized it, dragged it back, and proceeded to devour it at leisure. Apparently, it is only one or two days after hatching that the young get effective control of their movements, and they probably remain at least that long in the maternal brood pouch. When they are sufficiently active to make their exit they are equipped for the business of life. It was a matter of some surprise to observe how perfectly endowed the young are with the instincts of the parent forms. Their behavior in almost every respect seems exactly like that of the adults. The nest-building, movements within the nest, such as waving the antennules, retraction, reversal of position, springing out after food, jumping after passers-by, signs of timidity, as well as the general behavior outside the nest, are all carried on just as in individuals many times their size.

Regeneration.

No attempt was made to study the power of regeneration in Amphithoe, but it may be worth while to record a few observations that were made incidentally on the regeneration of the antennae. The antennae were removed in several specimens while studying the reactions of the animal to olfactory stimuli, and it was noticed that the regeneration of these appendages took place with considerable rapidity. Several observations were also made on specimens in which the antennae were found to have been accidentally lost. In a specimen (Fig. 2) observed August 13 the left antennule had been removed at the end of the second joint, and the right one at the end of the first. Of the second antennae the left member was off at the end of the fourth joint, which had a small, apparently regenerated knob at the end, and the right was gone at the end of the third and had a black tip. On August 16 the specimen moulted and the antennae appeared as follows: The right antennule had regenerated the last two joints of the peduncle and a flagellum of twelve joints; the left antennule also had completed its peduncle and regenerated a flagellum of seven joints, two of which were partially constricted in the middle and may have later become separated into two joints each. The second antennae had both completed their peduncles, the fifth joint in the right one being somewhat longer than in the left. The left antenna as a whole, however, was longer than the right, and its flagellum consisted of nine joints, while that of the right was composed of seven. How long the antennae had been injured when the observation was first made I cannot state, but it was certainly since the previous moult, so these appendages were probably regenerated in not much over a week and possibly in a less time. In another specimen first observed August 13 the left antennule was gone at the end of the second joint, and the right one at the middle of the second joint. The left second antenna was off near the end of the fourth joint, and the right one at the end of the third. After moulting, which occurred on August 14 or 15, the right antennule had a completed peduncle and a flagellum of six joints; the left antennule did not regenerate. Each of the second antennae had a complete peduncle, and the left one had a flagellum

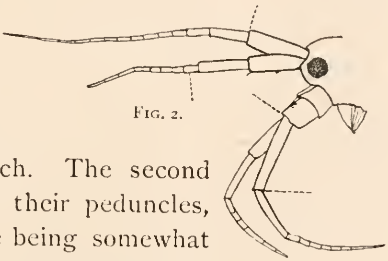

Fig. 2.

of three joints and a minute terminal fourth joint; the right one had a flagellum of four joints.

The Effect of Cutting the Animal in Two.

It has been found in many of the lower animals that, after removal of the posterior part of the body, the anterior portion manifests little signs of pain and acts as if no injury had been received. Many interesting cases of this kind have been collected by Dr. Norman in his paper entitled "Do the Reactions of the Lower Animals against Injury indicate Pain Sensations?"[1]

In order to see how the removal of the posterior part of the body affects the behavior of Amphithoe, I cut the animal in two just behind the third thoracic segment. In the posterior piece the thoracic legs moved but little, but the pleopods kept up their rhythmical beating for fully a half hour. The anterior part of the animal apparently suffered little discomfort, since, after the operation, it behaved much as if forming a part of the whole organism. The animal lay moving its antennules to and fro and making the usual movements of its gnathopods, just as an uninjured specimen would do. A piece of meat was brought near so that it was struck by the movements of the antennules; it was first swept a little nearer by the second antennae, and then quickly grabbed by the gnathopods, drawn in, and eaten, although the food could pass through only the small part of the alimentary canal that remained. Since this reaction in the normal animal is readily prevented by fear, and only occurs when the animal is hungry, it is somewhat surprising to find it performed after such a serious injury as the loss of the posterior half or more of the body. The animal behaves in such an apparently normal manner after this operation that it would seem as if the loss was scarcely felt. Owing to the loss of the posterior part of the body, many of the actions of the animal are naturally impeded or prevented. It cannot, for instance, get meat except when it is placed quite near, as it is unable to make its accustomed spring out of the nest. But in general, so far as the actions of the animal are modified, they are affected rather by the loss of certain organs than by any shock to the central nervous system. The loss of blood consequent upon the operation soon leads to weakness and finally death. Were it not for the profuse bleeding that occurs, the separated halves of the body could probably be kept alive for a long period.

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[1] *Am. Journ. Phys.* Vol. iii, p. 271. 1900.

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BIOLOGICAL BULLETIN.

NOTES ON THE HABITS OF PYCNOGONIDS.[1]

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THE Pycnogonids constitute a small and well-defined group, especially interesting on account of their peculiar and unique structure. In general they have been largely neglected by naturalists, especially as compared with some other groups, but they have received considerable attention from specialists, and several excellent monographs have appeared dealing with their structure and classification; in fact, all the literature of the group is very largely systematic or strictly morphological. The embryology has been worked out for some forms by Dohrn and Hoek, and in this country by Morgan,[2] who has also given an extended account of the metamorphosis of Tanystylum, and considerations on the phylogenetic position of the Pycnogonida.[3] The principal systematic work in this country has been done by Wilson,[4] who described some fifteen species found on the New England coast. Practically nothing has been written on their habits, which ought, it would seem from the isolated position of these animals, to offer some very interesting comparisons with those of other arthropods. An excellent opportunity for this work was offered during the past summer at the

[1] From the Zoölogical Laboratory, University of Michigan.

[2] Morgan, T. H., "A Contribution to the Embryology and Phylogeny of the Pycnogonids." *Stud. Biol. Lab. J. Hopkins Univ.* V., No. 1, pp. 1–76. 1891.

[3] Morgan, T. H., *loc. cit.*, and "The Relationships of the Sea-Spiders," *Biol. Lect. Marine Biol. Lab.* for 1890. Seventh Lecture, pp. 142–167. 1891.

[4] Wilson, E. B. (*a*) "A Synopsis of the Pycnogonida of New England," *Trans. Conn. Acad.* V., pp. 1–26. 1878. (*b*) "The Pycnogonida of New England and Adjacent Waters," *U. S. Fish Com. Rept.* for 1878, pp. 463–506. 1880.

Marine Biological Laboratory at Woods Holl, and at the suggestion of Dr. S. J. Holmes, to whom I am also indebted for much help and advice, I undertook to ascertain something of the habits and reactions of the forms found there. The present paper embodies some of these observations, which though far from complete seem to be of interest. I hope to be able later to supplement them by a more comprehensive account of the biology of the group. Much of this work is rendered difficult by the fact that the animals are not easy to observe under natural conditions.

As has been noted by Morgan and other authors, there are three species of Pycnogonids to be found at Woods Holl, representing as many genera. These peculiar animals may be found in nearly every collection of hydroids made from the piles or dredged up from the bottom, their long legs appearing to be hopelessly tangled among the stems of the hydroid as they kick slowly about in an aimless but persistent fashion. Perhaps the commonest of these, and by considerable the largest (extent 40 mm. to 50 mm.), is the dark purple colored *Anoplodactylus lentus* Wilson (= *Phoxichilidium maxillare* of Morgan and others), which is especially abundant in colonies of Eudendrium taken from the piles. A smaller species of a yellowish color, *Tanystylum orbiculare* Wilson, measuring about 7 mm. in extent, was found fairly abundant in a yellowish hydroid almost the exact color of the Pycnogonid. Anoplodactylus was on no occasion found among the light-colored hydroid, nor did I ever find a specimen of Tanystylum among the dark colonies of Eudendrium, where Anoplodactylus is fairly inconspicuous, though I have found the latter among the much lighter colored Bugula growing near the Eudendrium. I am not prepared to say that this is a case of color adaptation, as my observations were too limited to confirm this view, but merely throw out the suggestion for what it is worth. And it is worth remarking in this connection that the third representative occurring in this locality, *Pallene brevirostris* Johnston (=*P. empusa* Wilson), which is a slender whitish or more or less transparent form and is very hard to see for this reason, was found to be much more generally distributed than either of the other species, being found among both light and dark colored hydroids and algae. Pallene impresses one as the smallest form of the three owing to its extreme slenderness, though it is really almost twice the extent of Tanystylum, measuring some 12 mm. to 13 mm. across.

Swimming and Crawling Movements.

The activities of the three species of Pycnogonids under consideration are in a way directly correlated with their structures. Tanystylum, a short-legged and compact form, is very sluggish and inert; if placed at the surface of a dish of water, it kicks hardly at all, but sinks immediately to the bottom,[1] where it does not attempt to crawl but usually draws its legs together over its back and remains quiet. Pallene, on the other hand, under the same conditions does not sink to the bottom, but by vigorous kicking movements of its long slender legs remains suspended in the water, for a considerable time at least, its further movements being determined by the conditions, one of the most important of which, as will be shown later, is light. In the actions of Anoplodactylus there is great individual variation, but in general it may be said that they are intermediate between those of Tanystylum and Pallene. Some specimens sink almost at once to the bottom, where they rest in whatever position they may strike; others may crawl along upon the sand, or partly swim, touching the sand with only the tips of certain of the legs; or still others may swim entirely free from the bottom. As with Pallene, just what the animal does appears to depend largely upon the conditions. Most of my observations were made upon Anoplodactylus, for the reason that it was easiest to obtain and of convenient size for observing the movements in detail.

Before going further it may be well to give a brief explanation of the terminology which I shall use. Various authors have used different names for the seven pairs of appendages of the Pycnogonids, largely as they regarded them homologous to the appendages of the Crustacea or to those of the Arachnids. Dohrn obviates this difficulty by simply numbering them in their natural order, I–VII (Fig. 1).[1] For convenience I shall speak of the third pair as the ovigerous legs (these are absent in the female of Anoplodactylus), and of pairs IV–VII simply as the first, second, third, and fourth pairs of legs respectively, or, to distinguish them from the ovigerous legs, as the walking legs. Each of the walking legs is composed of nine joints (including the terminal claw), and all four pairs are essentially alike. As may be seen by reference to Fig. 2, the first three joints in Anoplodactylus are short and capable of comparatively little motion, while the fourth, fifth, and sixth joints are long, most of the movement of the leg taking place

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[1] In these experiments the bottom of the dish was covered by a layer of fine sand. The depth of water was usually about 5 cm. to 7 cm., though deeper water was tried with no difference in the results.

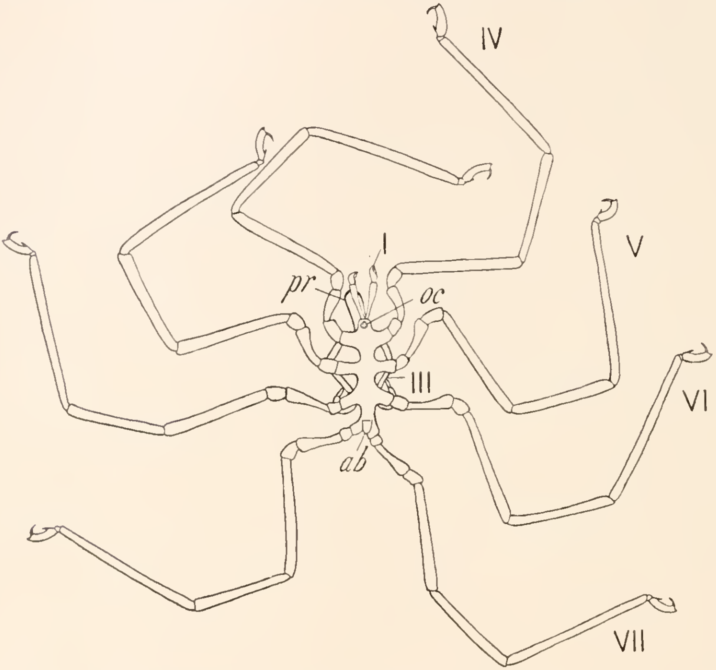

Fig. 1. — Male *Anoplodactylus lentus*, dorsal aspect: *ab.*, abdomen; *oc.*, oculiferous tubercle; *pr.*, proboscis; I, chelifori; III, ovigerous legs; IV–VII, walking legs. × 3.

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[1] The second pair of appendages, the "palpi," are absent in Anoplodactylus and Pallene.

in these. The articulations are so arranged that there is little chance for motion outside the vertical plane, the leg thus moving up and down in the same plane in which it extends from the body. There is, however, a possibility of movement of the leg backward and forward in the horizontal plane to a certain extent, this motion occurring chiefly in the articulation between the first and second joints. The principal movement of the leg, that is to say the movement in the vertical plane, when the animal is swimming free, is shown approximately in the diagram. Starting with the leg in the position shown at *A*, and considering only that portion distal to the third joint, the next movement is essentially a straightening out and raising dorsally of that part, bringing it into a position as shown at *B*. The leg is now extended still farther and brought downward to *C*, then inward, and at the same time flexed, to the original position, *A*. This serves to indicate the movements in a rough way, and it can be readily seen that so long as the specimen is free in the water and all the eight legs are working with this same treading motion, the tendency is to propel the animal dorsalward, that is, in a direction perpendicular to its dorso-ventral plane; the fact that the legs extend radially from the body (Fig. 1) would help to keep the animal going straight in this direction, *provided they all beat with equal force.* If the claw of any leg should grasp a solid object as it comes down from *B* to *C*, the movement *C–A* would pull the animal in the direction of that object.

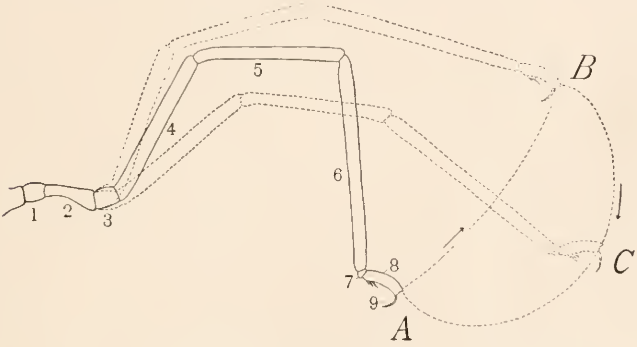

Fig. 2. — Diagram showing movement of leg in Anoplodactylus.

We are now in a position to examine more carefully the variations in the actions of the different individuals when placed in the water. As mentioned before, if the animal treads vigorously enough to overcome the force of gravity, it will swim; and so long as the body remains exactly in a horizontal position it will only move directly up or down according to the vigor of the strokes; but as soon as the body gets out of this plane the animal will progress through the water in the direction in which its dorsal surface is turned. For it to remain in a horizontal plane it is necessary that the legs should all beat with equal force; but as a matter of fact *the anterior legs beat oftener and with more vigor than the posterior legs*, thus raising the anterior end and tilting the animal backward. I did not make out any regular order of movement of the legs further than this, that the posterior legs seem to lack the vigor and strength and to be less under the control of the animal than the anterior pairs. A specimen which does not tread fast enough to raise itself from the bottom, or possibly one whose specific gravity is greater, crawls or walks straight ahead upon the sand, apparently, at first sight, much as an insect walks; but upon closer examination it may be seen that most of the movement is accomplished by the first pair of legs, assisted to some extent by the second, while the third and fourth pairs seem to be a hindrance rather than a help. By reference to Fig. 2 it can be seen how the anterior legs, by hooking into the sand, can pull the animal forward; while for the fourth pair to help in the forward movement it would be necessary for them to *push*, which would require a motion exactly the reverse of that which has been described for them when free from the bottom. Instead of this they drag along in a sort of helpless fashion, seeming to attempt the same movement as before, but hindered by striking the sand and by the forward movement of the animal as a whole due to the stronger anterior legs. A considerable backward and forward movement is now to be observed in the second and third legs, but this is probably also due to the pulling forward of the body after the legs are put down onto the sand, and not to a direct action of the legs themselves, the motion being allowed for, as before stated, by the specially arranged articulation between the first and second joints. In order to prevent the specimens from swimming while making these observations, and to force them to crawl, a small collar of tinfoil was clasped around the body between the second and third pairs of legs, care being taken that it was small enough not to interfere with the movements.

Reaction to Light.

As has been stated, one of the most important factors concerning the movements of the Pycnogonids when placed in the water is the direction of the source of light. If the dish containing them is placed near a window, the animals either swim or crawl quickly to the light side of the dish.[1] This fact has been noted by Loeb,[2] who says of Anoplodactylus : " Es ist wie die meisten frei beweglichen Bewohner der Oberfläche des Meeres positiv heliotropisch," and he also states that when the body was severed between the second and third pairs of legs, the anterior portion still reacted in the same way, while the posterior portion, which was comparatively inactive, moved independently of the light. These results were easily verified, and it was further ascertained that the oculiferous tubercle (Figs. 1 and 4, *oc.*) is the photo-recipient organ, for when this was cut off the animals failed entirely to show any response to the light. Although there are, of course, individual variations, it is surprising how quick this response usually is, especially if the dish is covered above and on the sides away from the window, so as to exclude all light from other directions. In the diffuse light three or four feet from a northwest window, lively specimens usually traveled an average of 12 cm. to 15 cm. in thirty to forty seconds. The response seems to be more pronounced when the light enters horizontally.

In moving towards the light the animals may adopt any one of the modes of locomotion previously described : (1) they may swim entirely free; (2) they may partially swim, kicking along on bottom with those legs that are down; or (3) they may crawl with all the legs on the bottom. The second method is the most common and the one in which the greatest speed is made. But the striking thing to be noticed is that in the first and second methods, those in which they swim or partially swim, the movement is backwards, or nearly backwards, while in the third method of locomotion, when they crawl on the bottom, they invariably go straight ahead, that is to say, with the anterior end directed towards the source of light. It thus appears that in moving towards the light they orient themselves differently, according to whether they swim or crawl; and, as I have shown, whether they swim or crawl depends directly upon the vigor or *rate* of the treading movement and not upon any difference in the *direction* of the stroke. The question naturally presents itself, Why should there be this difference in orientation in the two cases? In order to determine this, let us first consider specimens which are forced to crawl by being weighted with tinfoil. If an animal so weighted is placed in the water with its anterior end towards the light, it crawls directly ahead without turning; if its head is pointed in any other direction, it gains this same orientation by making a short circle, turning in the shortest direction towards the light. Now for any animal to walk in a circular path it is necessary for those legs on the outside of the circle to act with a greater force than those on the inside, and thus shove the body around; in the case of an animal orienting itself so as to head towards the source of light, this means that *those legs away from the light act stronger than those towards the light*, they being the legs on the outside of the circle which the animal describes in coming around. If this rule holds true when the Pycnogonid swims, and I see no reason why it should not, we have a simple explanation of its orientation with reference to light at all times. This can perhaps best be made clear by taking a particular case and following it through. An animal is placed on the bottom with the long axis of the body at right angles to the rays of light. This is represented in the diagram *A* (Fig. 3), in which we are supposed to be looking at one end of the Pycnogonid as it rests upon the sand. The arrow indicates the direction of the light. The animal at once begins to kick and the body is raised from the bottom, but since those legs on the side from the light (*a*) beat stronger than those towards the light (*b*), that side is raised more and the body is tilted so that the rays of light strike approximately perpendicular to the dorsal surface, as shown in *B*. Since the regular movement of the legs tends to propel the animal dorsalward, it moves toward the light. And now the fact that the anterior legs beat more efficiently than the posterior must be taken into account; this action tends to bring these legs uppermost, that is, around to the place of the legs (*a*) in diagram *B*. This is shown in *C*, where we see the animal from the side instead of endwise, as in *A* and *B*; in this position the posterior legs kick along on bottom. As a matter of fact the anterior legs seldom come entirely around so as to be directly uppermost, but only approximate that position, the third and fourth legs of one side or the other being the ones to touch bottom rather than the posterior legs; so that the animal does not move directly backwards but rather "cornerwise." If the movement is more vigorous, or if the light comes more from above, the animal may raise itself entirely free from the bottom, keeping, however, the same relative position.

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[1] This is not the case if there are hydroid stems or similar objects which the Pycnogonid can grasp. The tendency to cling to anything of this character seems to be stronger than the reaction to light.

[2] Loeb, J., " Bemerkungen über Regeneration," *Arch. Entwick-Mech.* Bd. ii, pp. 250–256. 1896–97.

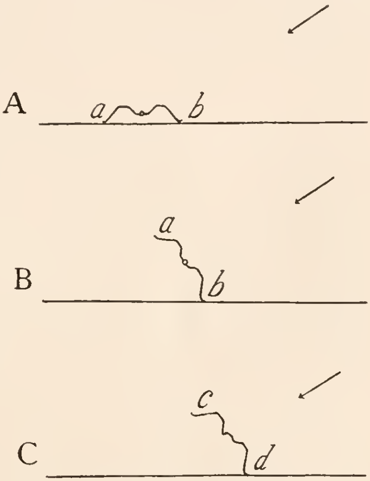

Fig. 3. — Diagram representing the reaction of Anoplodactylus to light.

If the orientation is different from the case given, it is easy to see how the same result is brought about. In a case where the animal is headed directly from the light, the anterior end has but to raise from the bottom to bring it into this position; and in the other possible case, when the head is directed towards the light, although the movements may be indefinite at first, it soon gets out of direct orientation and then turns around the shortest way, as in the first case.

Pallene shows even a more marked positive phototaxis than Anoplodactylus. If the light comes in nearly horizontally, it usually tilts over to an angle of about ninety degrees, or until the body is nearly perpendicular to the bottom, and moves quickly towards the light by a rapid movement of the legs. Pallene is much the better swimmer of the two and seldom moves along the bottom in the manner described for Anoplodactylus. So far as I could make out, the leg movement is exactly similar in the two species.

Transfer of the Eggs.

During the latter half of August the males of Anoplodactylus may often be found bearing the egg-masses upon their ovigerous legs. As a general rule, among Pycnogonids the eggs are gathered into little spherical or spheroidal balls strung along on the ovigerous legs, but in Anoplodactylus they are in more or less irregular masses through which both of the ovigerous legs pass (Fig. 4); their white color in this form, clearly offset by the dark body of the animal, gives them much the appearance of little bunches of wet cotton. Sars[1] says of the genus, evidently basing the statement on *A. petiolatus*, that there are "several globular egg-masses attached to the false legs in the male," and in his figure of this species there are five such masses shown on the right ovigerous leg. It is possible that in *A. lentus* the irregular masses may later

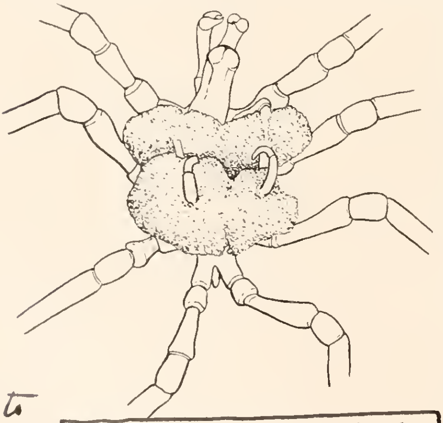

FIG. 4. — Male *A. lentus* from right side; walking legs removed. Reference letters as in Fig. 1.

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[1] Sars, G. O., "Pycnogonidea," *The Norwegian North-Atlantic Expedition, 1876–78. Zoölogy*, vol. vi, p. 25, and Pl. II, Fig. 2 *b*. 1891.

roll up into separate balls, for I have had opportunity to observe them for only a few days after they were laid; but this does not seem to me probable.

Cases of the males carrying the eggs are rare among animals and occur in widely separated groups; the male of the obstetric toad (*Alytes obstetricans*) winds the egg-strings about his body and carries them till the tadpoles hatch; the male of a South American frog (*Rhinoderma darwinii*) takes the eggs into his vocal sacs to develop; and the males of some of the Lophobranch fishes have brood pouches for the reception of the ova. In looking over the literature I have been unable to find any reference to this habit among the invertebrates, aside from the whole group of Pycnogonids. It seemed a matter of considerable interest to know just how such a seemingly intelligent act as the transfer of the eggs to the male takes place in animals whose movements in general seem to exhibit so low an order of psychic development, and I kept close watch of them with this point in view. So far as I am able to ascertain, the process has never been described, though Hoek[1] gives an account of the copulation in a European species as follows: "In regard to the way in which the eggs are laid, I had the good fortune to observe the copulation of a male and female *Phoxichilus laevis* Grube, when I was, last summer, in the zoölogical station of Professor H. de Lacaze-Duthiers at Roscoff. The eggs are fecundated the moment they are laid, and the copulation, therefore, is quite external, brought about by the genital openings of the two sexes being placed against each other. Half an hour after the beginning of copulation, the male had a large white egg-mass on one of his ovigerous legs, and about one hour later both masses were present." Only once, on August 16, at 6.15 A.M., was I fortunate enough to observe the pairing of Anoplodactylus. When first noticed both animals were among the hydroids; the male was clinging to the dorsal surface of the female and headed in the same direction. Both animals were kicking slowly in an indefinite sort of way, but gradually the male drew forward and, passing down over the anterior end of the female, came to lie beneath her, the animals being now headed in opposite directions and with their ventral surfaces opposed. The basal joints of the legs of the female were approximated below, with the mass of eggs between them. As the male came around below the female, the ovigerous legs, which are curved at the ends, forming a sort of hook (Fig. 5), fastened into the egg-masses, and as the animals separated pulled the eggs away with them. The masses did not pull away clean, but strung out more or less, leaving a very few eggs still on the female. For some time after they separated the male was observed to work the ovigerous legs slowly, the effect seeming to be to get the eggs more firmly upon them and into a more compact shape. The time from when the animals were first observed until they had separated was only about five minutes.

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[1] Hoek, P. P. C., "Report on the Pycnogonida, dredged by H. M. S. *Challenger* during the years 1873-76," *Challenger* Reports. *Zoölogy*, vol. iii, p. 131.

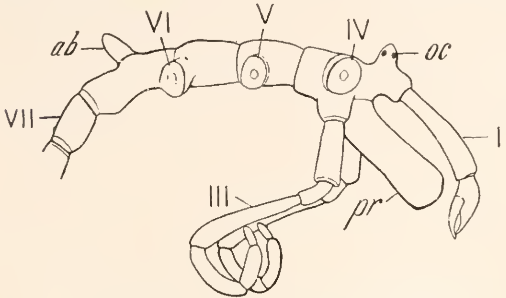

Fig. 5. — Male *A. lentus* from below, showing egg-masses on the ovigerous legs. Fig. 4.

Some of the males have but one egg-mass on the ovigerous legs, but more often there are two, as shown in Fig. 4. I am unable to say with certainty whether this means that the male takes the eggs from two females or that he gets them in two masses from one; but from the fact that in those cases in which they were examined the eggs in the two masses appeared to be in the same stages of development, I am inclined to the latter view. The genital openings are situated on the ventral side of the second joint of all four pairs of legs, and it is easy to see how the eggs of one female might gather into more than one mass.

SUMMARY.

1. The three forms treated are more or less adapted in color to their several habitats.

2. Swimming is accomplished by a treading movement of the legs, which tends to propel the animal dorsalward.

3. The stroke of each of the legs is the same in character, but is stronger in the anterior legs than in the posterior.

4. Crawling is accomplished by the same action of the legs as swimming, when the action is not strong enough to raise the animal from bottom. The anterior legs are most effective, pulling the animal forward; the action of the posterior legs is a hindrance.

5. Both Anoplodactylus and Pallene are strongly positively phototatic.

6. In *crawling* towards the light the animal proceeds with the anterior end in advance. If not oriented in this direction at first, it becomes so oriented by making a short circle, in every case towards the light. This means that those legs away from the light beat stronger than those towards the light.

7. In *swimming* towards the light the animal moves approximately backwards, with the anterior end somewhat raised. The amount it raises depends upon the activity of the individual and the slant of the rays of light.

8. This orientation is accomplished by the same actions that produce orientation when crawling, except that they are more vigorous, raising the animal from the bottom.

9. The transfer of the eggs from the female to the male is a comparatively simple process.

ANN ARBOR, MICH., Nov. 23, 1900.

EXPERIMENTS ON CUTTING OFF PARTS OF THE COTYLEDONS OF PEA AND NASTURTIUM SEEDS.

ABEGAIL C. DIMON.

THE experiments to be described were undertaken as bearing upon the general problem of the relation of food supply to growth. They were carried on under the guidance of Professor T. H. Morgan, to whom I am much indebted for aid and suggestions. The variations in food supply were produced in the pea and nasturtium, both dicotyledonous plants, by cutting off part of the cotyledons, thereby reducing the amount of food stored up by the parent plant for the use of the seedling. The questions that arise relate to the effect upon the size of the seedling, upon the differentiation of its organs, and upon the number and size of the component cells, caused by thus reducing the food supply. These questions may be answered from the results of the experiments.

As already stated, the pea and the nasturtium were the plants selected. Before deciding on them, however, the morning-glory, sweet-pea, radish, common bean, buckwheat, mustard, cucumber, and pumpkin were tested as to their suitability, by planting specimens of each with portions of their cotyledons cut off. It was found that the peas and nasturtiums, possessing large cotyledons, were more easily manipulated, and that their seedlings were hardier than those of the other plants. Their seedlings, moreover, grew rapidly, so that differences in the relative size of the plants were early noticeable and were well marked. Under favorable conditions, however, good results might be obtained from some of the other species, and it would be interesting to see to what extent they corroborate those from the two plants here discussed.

The seeds tested for availability, and subsequently all the pea and nasturtium seeds, were treated as follows : After they had been soaked in water for from twelve to twenty-four hours to soften them, their seed-coats were removed and, from some of the seeds, parts of the cotyledons were cut off with a sharp scalpel, while the others were left in their normal condition save for the removal of their seed-coats. The normal seeds underwent the soaking in water and removal of the seed-coats to make their condition like that of the others except in the one point of food supply. When thus prepared, all these seeds were planted on sawdust, which was kept wet during their growth. In the case of the pea and nasturtium, as soon as the plants from these seeds were well started a second lot of normal seeds, treated in the same way as the normal seeds described above, was planted. In a short time, usually about two weeks, the plants of the second lot were found to be about the same size as those of the first lot that had come from the reduced seeds. Sections of the stems were then cut freehand, and camera drawings made.

The amount of cotyledon cut off from the seeds varied very considerably, and was not in all cases quantitatively determined. The variation may be seen from Table I, where are represented the percentages by weight of the part left in the case of 57 peas and 35 nasturtiums. The seeds were weighed after the removal of the seed-coats and again after the removal of part of the cotyledons, and the percentages express the ratio between the two weights. The percentages in the case of the peas vary from 16 to 48, with more than half the individuals between 26% and 35%; those in the case

TABLE I.

PART BY WEIGHT LEFT, EXPRESSED IN PERCENTAGES.	NO. OF PEAS.	NO. OF NASTURTIUMS.	PART BY WEIGHT LEFT, EXPRESSED IN PERCENTAGES.	NO. OF PEAS.	NO. OF NASTURTIUMS.
10–15	—	2	31–35	14	5
16–20	10	9	36–40	5	1
21–25	8	9	41–45	3	1
26–30	16	8	46–50	1	—

of the nasturtiums vary from 10 to 41, with more than half the individuals between 16% and 25%, and nearly three-quarters between 16% and 30%. The chances, then, that from 65% to 74% of the bulk of any pea seed has been removed, are even; and the chances are three to one that from 70% to 84% of any nasturtium seed has been removed.

The variation in the amount of cotyledon removed appeared to influence the rate of growth of the seedling, but the number of plants of which a quantitative record of the development was kept was too small to justify an attempt to lay down a rule concerning the extent of this influence. The seedlings will therefore be regarded as belonging to only two classes, the normal and the dwarf, the latter composed of plants growing from seeds that have been reduced by removing part of their cotyledons. Plants of the two classes sprouted at about the same time, and for a short time the differences between them were not striking. As soon, however, as leaves began to develop, the normal seedlings shot ahead, surpassing the dwarf seedlings not only in size, but also in the number and size of their leaves. A comparison of two groups of pea plants, as given in Table II, from readings taken at three different times, shows the relative rate of development. The figures represent the height of the plants in millimeters and their number of leaves, while at the foot of each column is placed the average of the readings in that column. The readings from the dwarf seeds are arranged in the order of the fraction of the seed that is used for planting, and the readings from the normal seeds, in the order of the weight of the seeds, the smallest fraction and the smallest weight being at the top of their respective columns. The first period, two weeks after the seeds were planted, corresponds to an early stage of development; the second, five weeks old, is the stage just before the production of flowers by the normal plant; and the third period, nine weeks old, is the period of maturity, when the plants are bearing flowers and seeds. The letters *fl.* and the word *pod* in the column marked "leaves," mean that the plant against which they are placed has a flower or a seed-pod.

TABLE II.

	TWO WEEKS.				FIVE WEEKS.				NINE WEEKS.			
	Dwarf.		*Normal.*		*Dwarf.*		*Normal.*		*Dwarf.*		*Normal.*	
	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.		mm.	
a	17	5	35	11	22	9	77	23	35	—	95	fl.
b	10	—	29	11	29	9	70	19	47	—	89	—
c	16	5	32	13	35	15	86	27	44	—	108	pod.
d	22	7	32	11	26	9	77	27	35	—	102	fl.
e	26	11	32	11	54	20	89	27	70	fl.	117	pod.
f	26	9	38	13	47	17	77	27	63	fl.	95	—
g	29	9	41	13	60	21	92	28 fl.	77	pod.	108	pod.
Av. . . .	22	7	34	12	39	14	81	25	53		102	

From Table II we may collect several facts as to the relations in size and development between dwarf and normal plants. In the first place, the ratio of height of dwarf to height of normal, expressed in percentages, varies as follows: At two weeks, 65%; at five weeks, 36%; at nine weeks, 52%. The ratios between the number of leaves of the dwarf and of the normal are 58% and 56% for the two and five weeks old plants, respectively. No readings were made of the number of leaves at the third stage, because some of the plants had lost leaves, and the oldest leaves of all the plants were beginning to wither, so that the number had not as much significance as in the earlier stages. Similar measurements of height and number of leaves made on another lot of peas showed similar variations in ratios, so we may consider the readings here given as typical. The advantage in size, then, which the normal seedlings show in the first stage becomes more marked as the plants grow older, while the differentiation, so far as expressed by the number of leaves, though markedly greater in the normal than in the dwarf plant, does not increase much faster in proportion in the former than in the latter. The time of the first appearance of flowers is, however, more significant of the differentiation of the plant than is the number of leaves, for the latter may depend on the size of the plant, as well as on its degree of development. The time of flowering was unmistakably earlier in the normal plants, for the first flower appeared upon them at five weeks, whereas the first flowers appeared on the dwarf plants at eight weeks. At this time three dwarf plants blossomed, while during the same period five of the normal plants had borne flowers, of which three had developed into pods. These facts suggest the conclusion that the normal plants not only are larger than are the dwarf plants but develop more quickly than do the latter.

It remains, therefore, to be seen whether the foregoing conclusions to which the macroscopic examination has led are supported by the examinations of sections under the microscope. An examination of this sort may be looked to to answer the following four questions:

A. What is the relative size of the cross-section of a normal and of a dwarf plant?

B. What is the relation between the number of cells in a normal cross-section and a dwarf one?

C. What is the relation between a normal and a dwarf plant, as regards the size of the cells?

D. How does the degree of differentiation of the normal compare with that of the dwarf plant?

Attempts were made to compare sections of the dwarf seedling under the microscope with sections of a normal seedling of the same size, as a check, as well as with the normal seedling of the same age, but the only specimens of check seedling available for comparison were so much larger than the dwarf that allowance must in every case be made for a discrepancy. The cross-sections studied were all cut freehand from a level less than an inch from the ground in the growing plant.

The first question in the preceding paragraph relates to the relative size of the cross-section of the stem of the normal and dwarf plants. In the cross-sections represented by the figures the diameters of the peas have the relative values of 36 and 53, or the diameter of the dwarf pea is .68 as great as the diameter of the normal; the diameters of the nasturtiums have the relative values of 30 and 38, or the diameter of the dwarf is .79 as great as that of the normal. Cross-sections of the stem of the check plants have the values 46 and 33 for the diameters of the pea and nasturtium, respectively. The dwarf and normal plants were five weeks old and the check plant two and one-half weeks old. The plants selected for examination were typical ones, and the fact that the ratio between the diameters does not correspond to the average ratios between the heights given in Table II may be explained in two ways:

1. The ratio between the heights, as was seen, decreases with the increasing development of the plants, and the degree of development of the plants from which the cross-sections were taken was probably not the same as that of the plants measured in Table II.

2. The stem of small plants is always thicker in proportion to the size of the plant than that of large plants, so less difference in cross-section than in height is to be expected. Interpret this discrepancy as we may, the fact remains that the stem of a normal plant has a greater diameter than the stem of a plant sprung from a seed part of whose cotyledons has been cut off.

The ratio between the number of cells in a normal cross-section and a dwarf cross-section can be determined by counting the number in a definite sector of each. The results of counting the number of cells in a sector of 30° are : in the normal pea (Fig. 1), 410 cells ; in the dwarf pea (Fig. 2), 311 cells ; in the normal nasturtium (Fig. 3), 223 cells ; in the dwarf nasturtium (Fig. 4), 208 cells ; in the check pea, 404 cells ; in the check nasturtium, 219 cells. The dwarf plant has, therefore, decidedly fewer cells than the normal ; in the case of the pea .76 as many, and in the case of the nasturtium .93 as many. If the ratio between the number of cells was the same as the ratio between the diameters of the cross-sections, it would mean that the cells must be of the same size ; since the former ratio is larger for both pea and nasturtium, it means that the cells of the normal are larger than those of the dwarf plant.

The conclusion as to the size of cells in dwarf and normal plants may be confirmed directly by counting the number of cells in a definite area. Proceeding to do this for Parenchyma cells, the following results were obtained: In the normal pea (Fig. 1), 22 cells ; in the dwarf pea (Fig. 2), 43 cells ; in the normal nasturtium (Fig. 3), 21 cells ; in the dwarf nasturtium (Fig. 4), 32 cells ; in the check pea, 50 cells ; in the check nasturtium, 29 cells. The regions counted were all in the same part of the stem, and other counts were made that corroborate the figures here given. These figures confirm the conclusion reached in the preceding paragraph as to the size of the cells in normal and dwarf plants ; but in the case of the check pea plant it is found that the cells are smaller than those of the dwarf plant.

Though the statistics from microscopic examination that

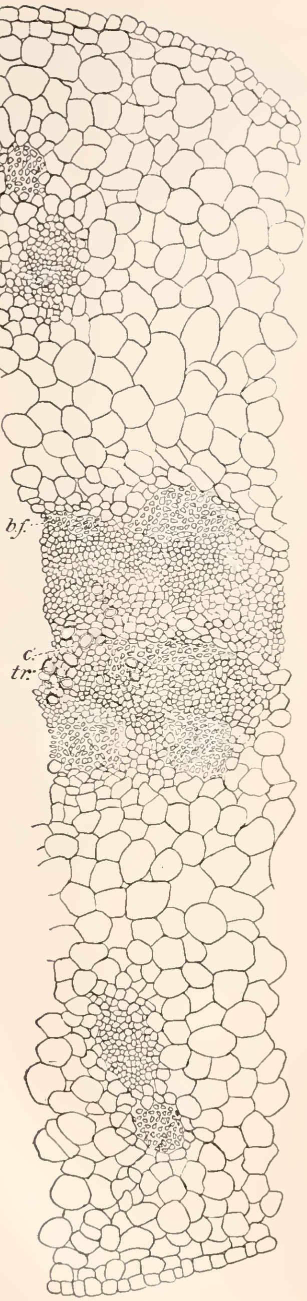

FIG. 1.

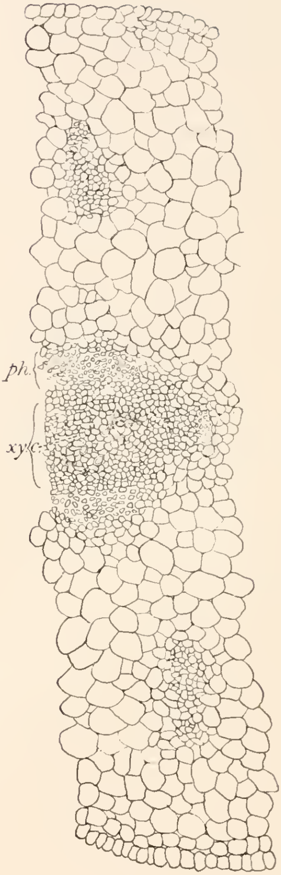

FIG. 2.

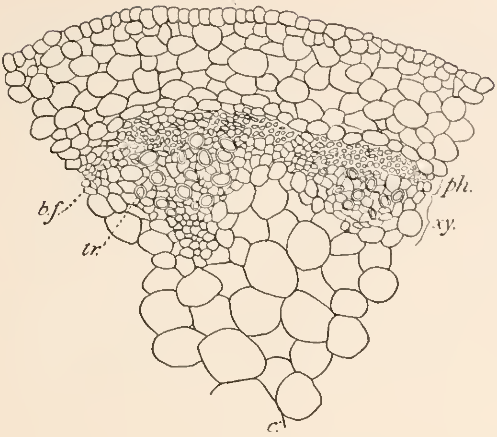

FIG. 3.

DESCRIPTION OF FIGURES.

b.f. = bast fibers.
tr. = tracheid.
ph. = phloem.
xy. = xylem.
c = center of stem.

FIG. 1. — Part of a cross-section of the stem of a normal pea seedling.

FIG. 2. — Part of a cross-section of the stem of a dwarf pea seedling.

FIG. 3. — Cross-section of a sector of the stem of a normal nasturtium seedling.

FIG. 4. — Part of a cross-section of the stem of a dwarf nasturtium seedling.

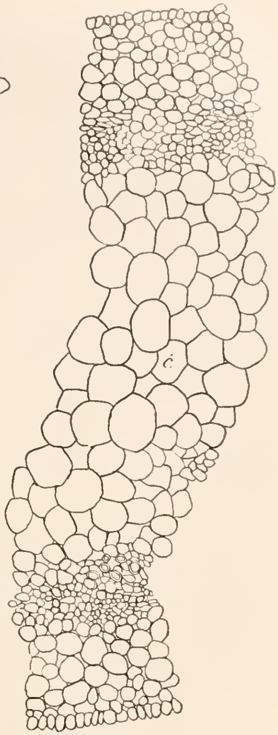

FIG. 4.

have been given and discussed were all drawn from three plants of each species, nevertheless that the general results are trustworthy is shown by another set of observations made on different pea plants. These gave the following results: Ratio of diameters of dwarf and normal plants, 111:62 (.56); cells in a given sector (7°), 165 in normal and 106 in dwarf (.64); cells in strips from center to circumference proportional in width to the size of the cross-section, 219 and 144 respectively (.66). The number of cells in a definite area was 29 in the normal and 44 in the dwarf. These measurements confirm those already discussed, for they show that the normal stem is larger in cross-section, is composed of a greater number of cells, and of larger cells than the dwarf.

The next question to be examined is the degree of differentiation of the various plants. This differentiation may be studied in the fibro-vascular bundles, where we may note the appearance of the bundle as a whole, and the development of its elements. The appearance as a whole is indicated diagrammatically by the text-figures, which show especially marked differences in the case of the nasturtium. In the dwarf nasturtium the fibro-vascular elements are arranged in separate bundles around a central pith, while in the normal plant the phloem of the different bundles has run together, making a ring around the pith, the xylem being still discontinuous. Bast fibers seem highly developed with thick walls, and the tracheids are large, numerous, and clearly differentiated in the normal nasturtium stem ; while in the dwarf stem the soft bast has just begun to show signs of thickening into fibers, and the tracheids are small, and comparatively few and poorly differentiated. In both specimens of pea the fibro-vascular elements are arranged in an aggregate in the center of the stem and in four small groups peripheral to the large group. These bundles are more distinct and woody-looking to the naked eye in the normal than in the dwarf plants, and both bast fibers and tracheids are more numerous and highly differentiated. The normal plant, therefore, is more highly differentiated than the dwarf, as well as larger.

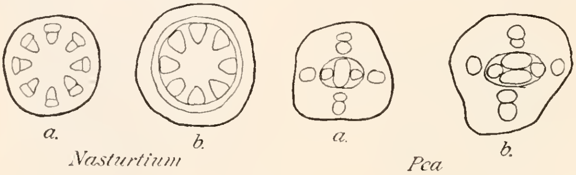

FIG. 5.

The conclusions reached from macroscopic and from microscopic examinations are then in accord with one another, and may be summarized by the statement that the removing of part of the cotyledons of a seed retards not merely the growth in size of the plant produced from that seed, but also its development. The plant, however, is not the counterpart of a younger normal plant, for it was found from comparing dwarf plants with check plants that the dwarf plant of a certain height was further developed than the check of the same height. The same point is illustrated by the fact that a full-grown dwarf plant is smaller than a full-grown normal plant, as is shown by the nine-weeks stage of Table II.

The effect, it would seem, of removing a part of its food supply from the seed is not merely a transient one, but is one that can be traced through the whole life of the plant, and even increases as the plant grows older. The amount of food supply in the cotyledons influences, perhaps, the early stages of growth, while as the plant increases in size it becomes more and more vigorous and tends to grow more and more rapidly; so that a plant that is given an advantage over its fellow at the start will increase this advantage during subsequent development.

VARIATION AMONG HYDROMEDUSAE.

CHAS. W. HARGITT.

The announcement of Bateson ('94), that "in the whole range of natural history there is no more striking case of the discontinuity and perfection of meristic variation than in the genus Sarsia, and the further proposition whether it is a mere coincidence that the specimens presenting this variation, so rare among the free-swimming Hydromedusae, should have been members of the same genus," directed my attention to this particular problem in conjunction with work upon this group of coelenterates which had engaged my attention for several years.

During the following years, therefore, collections of free medusae of several genera were made with a view to testing the problem raised by this observer. While as yet these collections are not extensive, except in a few genera, certain facts have been secured which may not be without value in their general bearing upon this as well as still broader problems of variation in general.

My collections have been restricted chiefly to the genera Eucope, Obelia, Margelis, Pennaria, Gonionemus, Coryne (Sarsia), and Hybocodon; the specimens of several others have been casually examined. Of the genera named, Obelia has not as yet been examined in sufficient numbers and detail to warrant any specific mention in this connection. And since these observations have been under way a paper by Agassiz and Woodworth ('96) on "Some Variations in the Genus Eucope" has appeared which so fully covers the facts involved in members of that genus, and are so coincident with my own, that no special details will be offered in connection with it, though the materials at hand are more abundant than upon any of the others.

What I shall have to offer in this paper, therefore, will be upon the other genera named, namely, Coryne, Gonionemus, Hybocodon, Pennaria, Nemopsis, and Margelis.

Coryne.

Of specimens of Coryne a comparatively few were available, though they were examined with unusual interest and care as belonging to the genus to which, apparently among the earliest, references to variation among Hydromedusae were made, and which called out the rather remarkable proposition of Bateson quoted in the opening paragraph of this paper. While the specimens were too few to warrant any definite conclusions, they nevertheless showed a most remarkable constancy in every morphological feature, not a single specimen exhibiting the slightest variation in any of the more conspicuous features, as tentacles, radial canals, manubrium, etc. If this constancy is as marked in different regions of distribution and for the large numbers cited by Bateson, it is not strange that he should refer to the matter in the terms quoted, as it would seem to be among the least variable of the free-swimming medusae of this group. It will at the same time show how very unsafe must be any such conclusion taken from so limited a range of observation.

Hybocodon.

Of the genus Hybocodon Ag. the number of specimens at my command has likewise been somewhat limited, slightly less than two hundred, still they have been sufficient to show some variation in certain features. This genus was instituted by L. Agassiz ('**62**), under which he included a Hydromedusa of very unique characters (*cf. Contribution to the Natural History of the United States*, Vol. IV, p. 243), one of which is the proliferous budding of medusae from the hydranth, which in turn give rise to secondary and many later specimens by a similar process of budding. (*Cf. op. cit.*, Pl. XXV, Fig. 13.)

The specimens which came into my hands were all preserved in formalin and had in consequence suffered considerable distortion in the process, whereby minute variations of organs often became difficult of detection, yet I was able to demonstrate a fair degree of constancy in the general form of the medusa, its radial canals, tentacles, etc. I desire to direct attention to the number of tentacles. As stated by Agassiz, there seems to be a single long tentacle arising from the margin of the bell at the terminus of one of the radial canals, from the base of which arose later the proliferous medusae-buds, as shown in the figure already cited. From these secondary medusae other tentacles arose, giving to them the exact morphological equivalent of the primary or mother medusa. Hence, as several of these proliferous specimens budded off from the base of the primary tentacle, several tentacles would come to be clustered near the same point, giving the impression of a bunch of tentacles of the same nature. In several specimens in which the medusae-buds had not yet appeared, or could be detected as mere papilla-like bodies, these secondary tentacles were nevertheless well developed, and of a length frequently equal to that of the primary one. Now whether this be a variation, or whether it may not be rather fundamental, arising as a source from which the medusae are to spring, may perhaps be an open question, to be settled by a more critical examination of their development. Proceeding on the assumption before stated, I venture to cite it as a case of variation, though it may later be found to be rather the normal process.

The rather unusual character of this medusa, both in its origin and proliferous progeny, led me to suspect that it might exhibit more than the usual phases of variation ; but in this I have been disappointed, except in the point just cited, — its constancy in almost every morphological detail being quite marked. As stated, however, in connection with observations upon Coryne, the limited number of specimens examined, and furthermore their distortion due to preservation, are barriers which should suggest reasonable caution in the formulation of any conclusion.

Pennaria. — Of this medusa I have had an almost unlimited number of specimens, having collected them during three years devoted to the embryology of the species common in the waters of the Massachusetts coast, — P. Tiarella McCr. A critical study of these medusae is, however, rendered difficult and tedious owing to their minuteness and form. In size they are only about .8 mm. in diameter by about 1.5 mm. in length. The highly oval form renders difficult a study of the aboral surface and the junction of the radial, or chymiferous canals, — a point of considerable variability in many cases in other genera, notably Gonionemus, to be noted later. In a study of their morphology Smallwood ('99) has pointed out the variability in the structure and development of these canals. He has shown that in a considerable proportion of specimens there is a tendency to atrophy both in the radial and circumferential canals, especially the latter. These changes are not evident in a surface study of specimens, the pigmentation which marks their course being fairly constant. The principal variation to which I desire to direct attention in this connection is a physiological one, *viz.*, a rather marked variation in habit and activity. I have discussed elsewhere this feature ('00) and need only refer here in brief to those observations. As there pointed out, there seem to be two rather distinct features of habit; namely, a rather deep-water habit upon rocks, seaweed, piles, etc., and a surface habit upon eel-grass or similar support, which serves to bring the colonies to the surface, thus often in a low tide exposing them directly to the action of the midsummer sun and temperature.

Associated with these differences are correlated variations in the form and color of the colonies, or, as Bateson would designate them, "substantive variation." The surface or eel-grass varieties exhibit more distinctly the pinnatifid character which marks its specific peculiarity, due doubtless, in part at least, to the prone or floating disposition of the colonies. Associated also with this is the much higher coloration so conspicuous in these specimens, a variation extending not only to the perisarc of the colonies but also to the medusae and the eggs, which are rather bright orange, while those taken from the deeper waters are a pale, creamy white, with the slightest trace of pink in many cases.

Of the further physiological differences one of the most marked is that of the relative activities of the medusae of the afore-mentioned varieties; those of the surface habit exhibiting a much greater degree of activity and other vital phenomena. These, as previously pointed out, are extremely active, being liberated from the hydranths promptly upon maturity, swimming with great ease and freedom, and discharging the sexual products with great promptness. On the other hand those of the deep-water habit are passive, or even sluggish, — in many cases the medusae never becoming free from the hydranth, — discharging the sexual products with much less regularity and ease, and dying very soon after. These medusae are short lived at best and never increase in size after liberation from the hydroid. I would suggest the probable correlation of some of these features of variation with the degenerative tendency shown in both structural and physiological variations already noted, especially in the atrophy of the chymiferous canals.

A histological study of the tentaculocysts likewise shows degenerative tendencies, as does also the very rudimentary condition of the tentacles, which are barely distinguished as bud-like protuberances upon the margins of the bell.

In connection with previous work upon the development of Pennaria, attention was directed to variation in the rate of cleavage and subsequent development. This would seem to be a matter of considerable interest in connection with the fundamental problems of physiological variation. It is well known, of course, that cleavage is a phenomenon subject to considerable variation as to rate, due to variable conditions, and to some extent independent of sensible differences of environment. It seems to me, however, that in the case of Pennaria there are presented such marked extremes in this respect that it may well be considered as in some measure correlated with other features of physiological variation.

The variable rate in the later phases of larval development is also worthy of note. From data obtained during three summers of observation the range of time involved in the larval history varies from about two days up to about two weeks.

While in most cases these observations were made upon specimens under artificial conditions, namely, aquaria of variable sizes, etc., still the variations occurring were exhibited by larvae under identical conditions, such as they were.

Under the head of abnormalities in immediate connection with these observations, attention was also directed to certain variations in the morphology of the larvae and early polyps. Among these may be mentioned

1. Twin-planulae, — planulae with bifurcated ends, irregular bud-like outgrowths, etc. (*Cf. op. cit.*, Figs. 4–6, and 8, Pl. 1.) It was suggested that they were probably due "to the intrinsic prepotency of hydroids to bud and branch." While this is probably an explanation of the facts, that they exhibit interesting variations from the ordinary is not discredited on that account.

2. Attention was also directed to an interesting polyp form (*op. cit.*, Fig. 1 of text), which presented so marked a variation as to give rise to some doubt concerning its Pennarian affinities. In view of the rather large range of variability exhibited by the medusoid and larval persons already considered, I am still convinced that this is only a further illustration of the same principle. Indeed, I have during the present summer observed in other polyps reared under similar conditions the same variation from total annulations to less and less degrees. A few additional annulations of the hydroid perisarc is matter of no special surprise. A complete annulation of the early and plastic colony, while quite unusual, need not be regarded as improbable or especially strange.

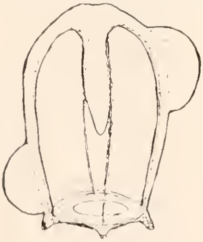

FIG. 1.

Another feature which may perhaps rather be designated as a monstrosity, or incidental excrescence, may be noted in this connection; namely, certain wartlike or pustular vesicles which often appear at various points on the exumbrella of the medusae. These are fairly represented in Fig. 1. The figure indicates relative positions where they are most likely to occur, though in no case have I noted more than one upon a given specimen. . . . A similar structure is referred to by Agassiz ('65) and explained as due to distortion caused by the pressure of the ova within the bell. This I am convinced is a mistaken view, for I have noted it upon specimens both living and on those killed and preserved in formalin, in specimens with and without eggs. It seems, moreover, to be wholly restricted to the outer ectoderm only, in no case involving the inner ectoderm of the subumbrella. There is nothing indicative of the cause or character of these excrescences. Whether they are permanent or merely transient features I am not able to say, the short-lived condition rendering any determination difficult if not impossible.

Nemopsis.

Through the courtesy of Mr. Strong I had the privilege of examining a small collection of about one hundred specimens of Nemopsis Bachei taken in the tow off Tarpaulin Cove. The variations here seemed quite as evident as in Eucope and Gonionemus. Here again the variable features included radial canals, manubrium, gonads, and tentacles. Fully five per cent showed some feature of variation. About two per cent had but three radials and three gonads. One of these showed a definite correlation, including all the organs named above. One, however, of the trimerous forms had a fourth sensory bulb and tentacles, though these were less prominent than were the other three sets. The oral tentacles likewise shared in the correlation.

One specimen was a symmetrically pentamerous form with a perfect correlation of all the organs under consideration. Another specimen was quite as symmetrically hexamerous.

Several other specimens exhibited apparent symmetry in the number of gonads. Frequently one of the series showed the gonad of one canal very unequally developed as compared with the others. But while approaching sexual maturity, and in many cases fully so, it is of course impossible to say with certainty that the short gonad might not have shown further development with age. In any case it certainly showed variation as to *development.*

Concerning variation in the number and order of tentacles it is difficult to determine definitely, since in Nemopsis they constantly increase in number as the medusa grows, much as in Margelis. So while there appears to be considerable variation in the number and arrangement it may be rather due to variable development than to any actual meristic variation. The same may be true as to the order of appearance. The paired, capitate tentacles at the apex of the bulb appear uniformly first and seem to be fairly constant. The latter filamentous tentacles appear to arise in pairs successively toward the margins of the bulb. Since I was not able to follow this development in the stages of growth of the medusa it is impossible to determine definitely this point. So while there is upon the preserved specimens considerable want of symmetry in this respect, yet it may be due in part to slightly variable rates of development. No constancy was apparent in the matter, and it would seem therefore to be physiological rather than morphological.

Similarly there was apparently some variation as to the number and distinctness of the otocysts upon the sensory bulbs. Normally there is a single eye-spot at the base of each tentacle. But in many cases they were apparently absent. And while it is not impossible that they had been rendered indistinct by the formalin in which they were preserved, still it remains quite certain that marked differences were distinguishable among various specimens of similar size and preservation, and perhaps only critical histological examination will be adequate to finally determine this point, and this I have not been able to make.

Margelis.

Of these medusae more than five hundred specimens have been examined, most of which were quite young, having only the four pairs of marginal tentacles and four unbranched oral tentacles and measuring only about 0.5 mm. in diameter. Typically this medusa may be characterized as having a high hemispherical bell, four radial canals, at the distal or marginal ends of which four clusters of filiform tentacles arise. The bell is thick, velum not specially prominent. Manubrium subconical, bearing four oral tentacles which divide dichotomously into small clusters of tentacles. Figs. 2–4 give good general impressions of the animal. The very minute size made necessary the constant use of the compound microscope in all the examinations.

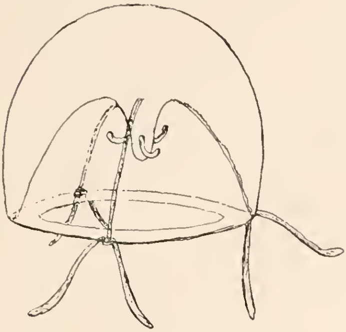

Fig. 2.

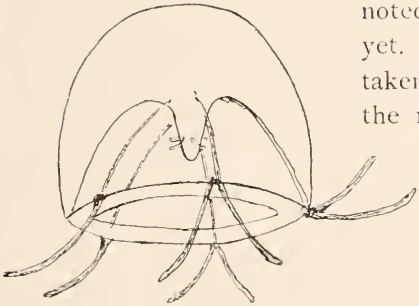

Fig. 3.

In general this medusa seems to be fairly constant in form, color, and organic symmetry. Only the radial canals, and tentacles — marginal and oral — were noted, no gonads being present as yet. Of the tentacles note was taken of the number of groups, the number of individual tentacles increasing in these with age. The total of all sorts of variations noted hardly exceeded two per cent.

The variation in radial canals was usually correlated with that of the tentacles. In Fig. 2 is shown a trimerous specimen, in which there was a perfect correlation of all the organs, including oral tentacles (several specimens noted). In Figs. 3 and 4 is shown a pentamerous form, in which there appeared little meristic correlation. For example, it will be seen that while five sets of tentacles correspond with the five radial canals, two had but a

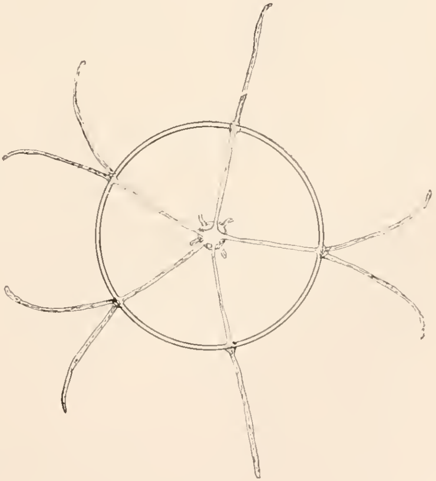

Fig. 4.

single tentacle each, while the other three sets contained the normal (at this stage) number, two. A point not shown definitely in the figure is the fact of only four oral tentacles.

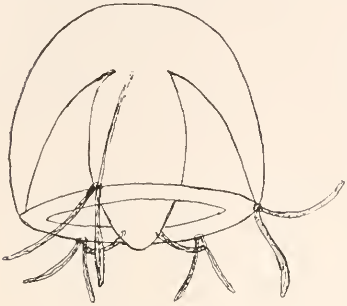

FIG. 5.

In Fig. 5 is shown a specimen with monstrously developed manubrium, protruding beyond the velum, and provided with but two tentacles. In several specimens similar enlargements were noted, though not so pronounced as in this, except in certain cases, apparently pathological, in which the entire bell was evaginated and greatly shrunken, with the manubrium greatly enlarged. No account was taken of such specimens, for they were evidently due to conditions other than those of health. It may be noted in this connection that several specimens were found exhibiting similar vesicular or pustular enlargement to those observed in connection with accounts of Pennaria. Here is further evidence, if such were necessary, that these enlargements, resembling distortions, could not have been produced by the enlarged gonads, for in the species under consideration the sexual organs were as yet undeveloped.

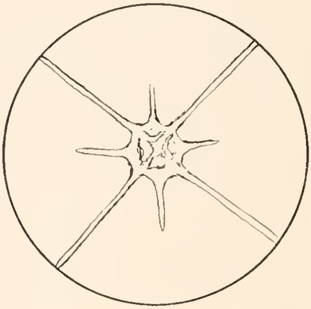

FIG. 6.

In Fig. 6 is shown in diagram an aboral view of a condition found in several specimens, in which there seem to be secondary, peripherally directed radial canals, extending nearly half-way over the bell. As this medusa has normally but four such canals this is a well-marked case of variation in the direction of a condition quite common in Rhegmatodes and many trachomedusae. I regret that none of the specimens to which I have had access are of approximate maturity so that such incipient variations might be traced forward to their perfection, in order to ascertain whether additional sets of tentacles, etc., would be found correlated therewith.

The origin and symmetrical interpolation of such secondary canals is very different, it seems to me, from that arising from the bifurcation seen in Eucope and Gonionemus, though their function in the economy of the organism may be the same.

Only one phenomenon more will be discussed in this connection, namely, that of double or twin medusae, which is shown in Fig. 7. Only one specimen of this character was found in the entire lot. In every respect — size, general form, organic relations, etc., the double feature alone excepted — the specimen seemed fairly normal, having this one further feature, that one was a trimerous specimen and that one set of tentacles contained three as against the two each of the other sets. The furrow-like depression along the line of union presents the aspects of late coalescence similar to that involved in artificial grafting (*cf. Biological Bulletin*, Vol. I, p. 41).

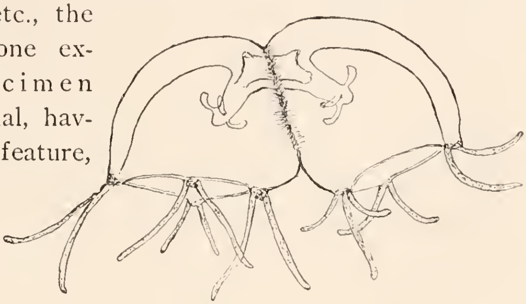

Fig. 7.

But a more critical examination shows that the union is much more profound, involving the gastric cavity and therefore the whole chymiferous system.

The specimen was not seen alive, and hence nothing can be said as to coördination of physiological activities, mode of progression, etc. But from what has been proved from experimentation on these points (*cf. op. cit.*) it may safely be inferred that similar coördination at least prevailed in such a case as that under consideration.

Podocoryne.

Of medusae of this genus I have had at my command only a few more than one hundred specimens, a number too small to warrant any formal deductions, but taken in connection with others it will not be amiss as showing the facts of variation pertaining to this genus.

Like Margelis this medusa is quite small when liberated, indeed never attains a size of more than 1 mm. in diameter, so far as I am aware. In general, it is similar in organization to Margelis, has four radial canals and four primary tentacles, between which a second series of the same number soon arises. Like Margelis there is apparently a fair degree of constancy in meristic features. Three trimerous specimens occurred in the lot, which comprised the extent of variations along this line. There were, however, considerable variations of size, form, etc., which increased the total to at least five per cent. It must not be forgotten, however, that the extreme minuteness of specimens, necessitating the constant use of the microscope, might easily involve an oversight of specimens in sufficient numbers to materially raise this ratio of variation.

Gonionemus.

It is among the members of this genus that I have been able to work out the most numerous and detailed series of variations. The total number of specimens of Gonionemus examined during the progress of the work was more than two thousand. (Of the first series studied no record was kept.) While smaller than the number of Eucope, to which reference has been made, the number is yet sufficiently large to insure against an unusual per cent which might be due to local or other incidental influences. Moreover, the collections were made during three summers, and by several collectors, so that the results obtained may be considered as closely approximating the actual state of variation now under way. It is a pleasure to acknowledge in this connection courtesies from Messrs. Coe, Parker, Perkins, Gray, and others for permission to examine collections of these. medusae made by them, which has facilitated my work.

In these studies attention has been directed chiefly to the following structures: 1, radial canals; 2, gonads; 3, manubrium;

4, tentacles; 5, otocysts. Other features of subsidiary nature will have mention in their appropriate places.

It ought to be stated in passing to details and tabulation of results that only in the matter of radial canals and gonads have the entire two thousand specimens been examined, and not all of those with equal detail in each case. In number and variation of tentacles, spurs, anastomosing of canals, etc., the tabulations were limited to one thousand; and in the case of otocysts to less than one hundred, a reason for which will be given in the appropriate place. I may also state that owing to the insignificant sexual differences, usually requiring microscopic examination to certainly decide, no effort has been made to determine the relation of variation to sex.

Gonionemus is a rather well-defined trachomedusa, first definitely described from the Atlantic coast by Murbach ('95). It is characterized by the typical four radial canals, which have the folded gonads suspended along their under surfaces, cruciform manubrium, as seen in transverse section, normally with four oral lobes and sinuously folded lips. Tentacles are numerous and similar, each characterized by a suctorial bulb near the tip, beyond which it often makes a sharp bend.

These medusae abound from June to October in a small pond near the Marine Laboratory, known as the "eel pond," which communicates with the open harbor by a narrow inlet. Lately they have been taken in the outer harbor, though in small numbers. I may mention this fact of the localized habitat, since it may well be a question whether its peculiarity may not be an important factor in the physiological aspects of the variations to be considered.

Taking up now the consideration of the several points in the order given, attention will first be directed to the

Radial Canals.

On this point an estimation of the ratio of numerical variation based upon fifteen hundred specimens gave nearly five per cent (4.82). On the matter of their form or disposition, *i.e.*, whether in their course to the marginal canal the bells were divided into symmetrical segments, the results showed no less than thirty per cent of variation. On the variations shown in their aboral confluence, or union with the gastric pouch, the calculations gave 14.4 per cent.

Of the entire number examined only a single specimen was found having but two radial canals and two gonads. These were at an angle of 180 degrees, *i.e.*, dividing the bell into two symmetrical halves, as shown in Fig. 8, excepting alone the relative number of tentacles upon each half. One other specimen, however, was found having only two canals, similarly disposed and with a similar number and disposition of gonads; but in it there was a rather evident rudiment of a third springing from the peripheral end of one of the canals, thus destroying the bilateralism characteristic of the first.

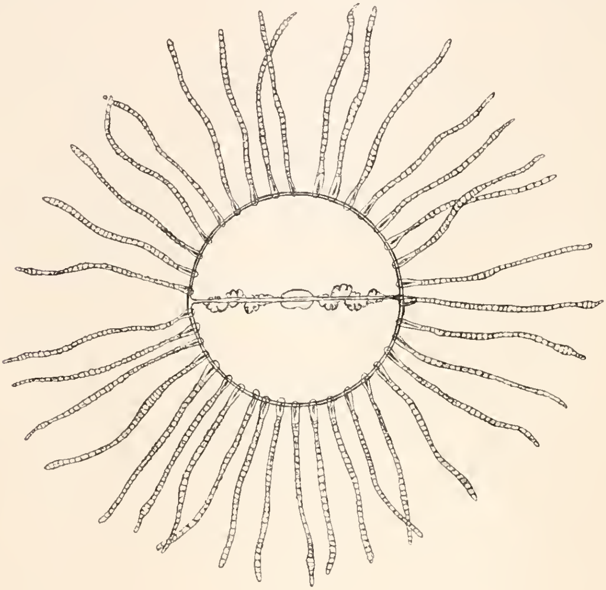

Fig. 8.

The largest number of canals found was six. This, while much more common than those with two, was much less so than specimens with three and five. Seven specimens in all were found having this number, and in one of these the sixth was due to the evident forking of one of the five apparently primary canals, which divided the bell into approximately pentamerous segments, as shown in Pl. III, Fig. 12. In another there was a very evident forking of two of the four primary canals, as shown in Pl. III, Fig. 9; for while the bell was divided into hexamerous segments, the manubrium was symmetrically tetramerous. In every one of the other five hexamerous specimens the canals converged at the aboral pole in a perfectly symmetrical way, though the hexamerism extended to the manubrium in only two specimens, and in these only the basal portion or gastric pouch was strictly hexamerous. In at least two other of these hexamerous specimens the oral lobes of the manubrium were four (*cf.* Fig. 9).

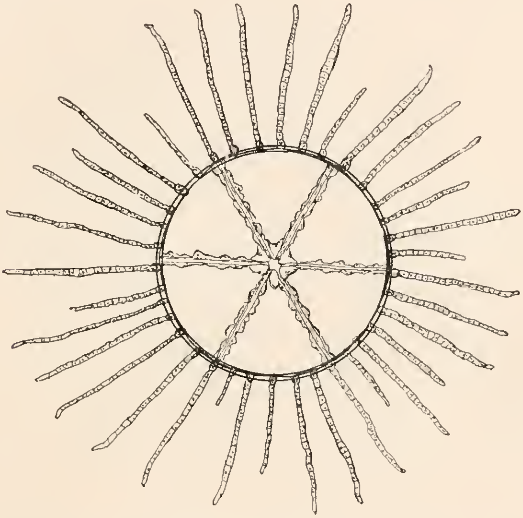

Fig. 9. — Hexamerous specimen showing pentamerous stomach and varying size and distribution of tentacles.

Of specimens having three and five canals there were by far the larger number, with the preponderance slightly in favor of the trimerous variety, but not sufficiently so to warrant any conclusions as to the question whether the course of variation was toward a trimerous rather than a pentamerous condition. Of those with three canals there were twenty-one specimens, while of those with five there were eighteen specimens. Of those making up the total of seventy-two specimens there were twenty-five specimens from distinctly tetramerous forms, having short forks less than one-third the length of the entire canal, or of such other variable aspects as to warrant their inclusion under this head. Pl. II, Figs. 6–9, and Pl. III, Figs. 1–5, will best illustrate this point.

It now remains to consider somewhat more in detail the individual variations exhibited by these several types. Directing attention first to those illustrated by the figures just cited, it may be seen that the variation here seems to be in several directions. (1) Atrophy, as shown in Pl. II, Figs. 4, 6, 8, and 9. In all these the evidences of degeneration are quite clear. First, in Figs. 6 and 9 there is the atrophy of the connection of one of the canals with the gastric pouch and the correlated reduction of the fourth gastric pouch and the further failure of the obsolescent canal to develop its usual gonad, the merest rudiments of which are apparent. Furthermore, in the same figure there is shown a still further atrophy of a second canal, which extends only about halfway to the margin, and correlated with that fact is the associated imperfect development of its gonad. A still further illustration of this degenerative

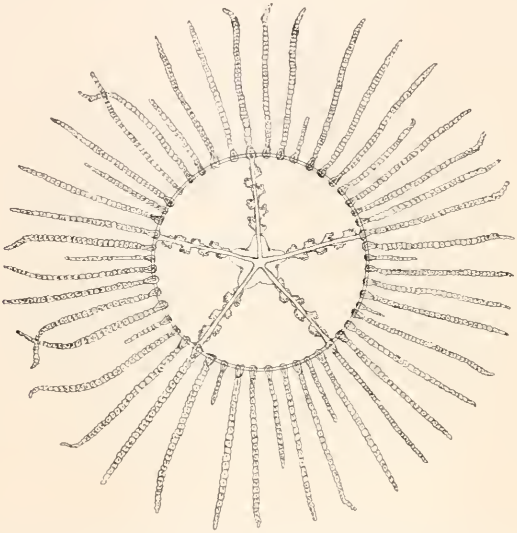

FIG. 10. — Symmetrically pentamerous specimen, but with the several series of tentacles appearing at irregular intervals.

tendency is shown in Pl. III, Fig. 6, where only the vestige of the fourth canal is shown, the reduction in extent of two others, with the further correlation of the evidently bilobed condition of the gastric pouch.

(2) Asymmetry. This is more or less consequent upon the atrophy already noted, as will be seen from a comparison of the figures just cited, and involves to a certain degree the entire organism, gastric and oral symmetry, no less than that of the bell and tentacles. Some further reflections on these lines will more naturally come up in connection with later discussions.

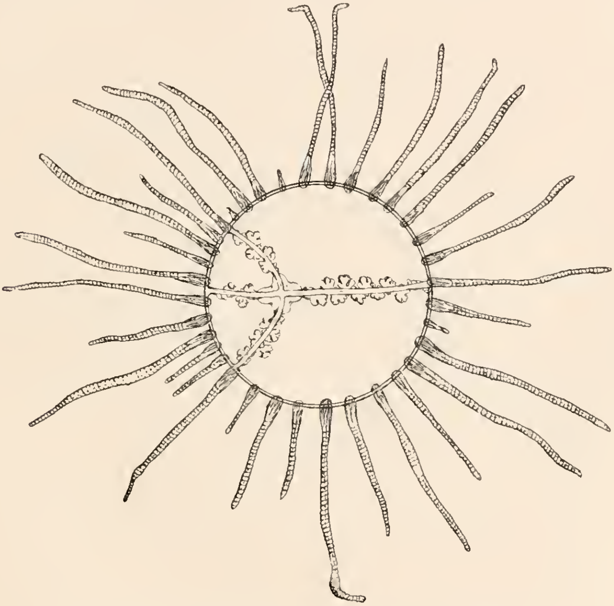

Fig. 11. — Tetramerous specimen of very unsymmetrical type.

Another type of variation is shown in Pl. III, Figs. 1–5. In these specimens, while the tetramerous type is more or less evident from the number of canals, gonads, or gastric pouches, still there is a rather definite tendency toward a trimerous aspect of the medusa as a whole, so far as the segmentation of the body is concerned. In Fig. 1, while there is a clearly tetramerous condition exhibited which extends to the several organs involved, there is yet such an approximation of those marked *a* and *b* as to leave the bell and number of tentacles in a closely trimerous symmetry. In Figs. 3–5 this evolution of trimerism is so evident that it would seem to point toward a preponderance of variation in this direction. As, however, will be seen later, facts of a very different kind seem to point as clearly in the opposite direction. It may as well be pointed out in this connection that the loopings of canals shown in the figures under consideration are variously simulated by structures shown in Pl. II, Figs. 1–4, and 10. A critical comparison however, while showing many unusual features in these latter structures, will probably demonstrate their fundamental likeness; but this will be considered later.

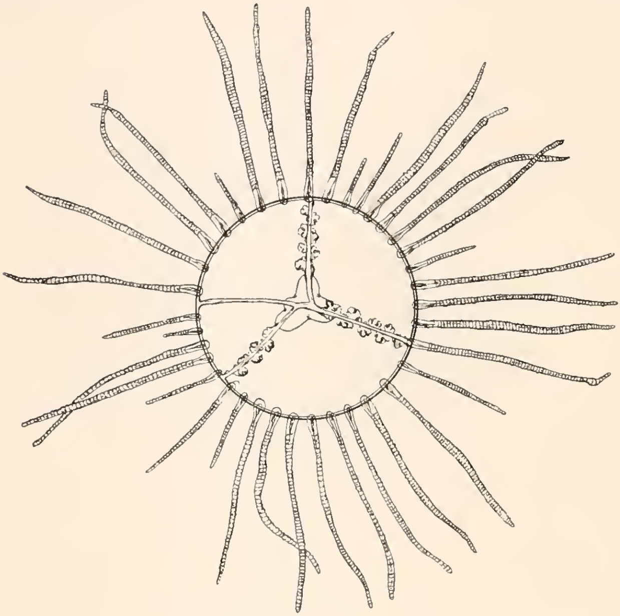

FIG. 12. — Specimen of very unsymmetrical character.

We may next consider a type of variation fairly illustrated by Pl. III, Figs. 9 and 11. As will be seen from a glance, there is here exhibited a clearly defined tendency toward an increase in the number of canals, hardly less marked than that of decrease just considered. Indeed, specimens with bifurcated canals of this character were rather more common than those of the last type, a fact, when taken in connection with the closely similar number of distinctly pentamerous forms, of great importance as showing that in neither case are we warranted in concluding that the course of variation is in one direction rather than in another. In order to reach anything approximating conclusiveness on this point a larger number of specimens studied through a successive series of years would be necessary. As I have already intimated, the collections forming the basis of the present discussion of this genus were made during at least four years, and while I have not made this a matter of critical comparison, it has not been at all apparent that during this period there has been any appreciable ratio of difference.

Passing now to the consideration of other aspects of variation evident in the canals, attention is next directed to their morphology. As is well known, the chymiferous canals in medusae are tubular structures of fairly constant size in members of the same species and of similar sizes, and their courses are usually direct from the center to the margin in most of the Hydromedusae. As Agassiz and Woodworth ('**96**) have shown in the case of Eucope, however, there are not a few departures from this rule. The same is true of Gonionemus, as a glance at Pls. I and II will demonstrate. Not only does the diameter of the canal vary greatly in many specimens, which is of only incidental concern, but in many cases, as in Pl. I, Figs. 9, 11, and 12, various loops and diverticula in the form of spurs are formed at various points and at various angles along their course. These are of varying sizes, lengths, etc., and were found on between one and two per cent of all the specimens examined. In the paper just cited the authors suggest in these facts a possible simulation of a condition "characteristic of the Discophores" (p. 122). Whether there is in these structures anything more than simulation or parallelism as compared with the Scyphomedusae, I shall not at present discuss.[1] As compared with the typical canal, however, there seems to me to be little doubt that they are fundamentally of similar origin and function. While in many cases there are extremely small serrations of the canal walls, in other cases (Pl. I, Figs. 1 and 2; Pl. III, Figs. 4 and 5) they are more prominent, even occasionally forming anastomosing connections between adjacent canals. Similarly the loops already referred to are probably in most cases anastomosed spurs.

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[1] It may not be amiss, however, to state in this connection that in Rhegmatodes there is a much more evident correspondence or resemblance in this matter than in either Gonionemus or Eucope. While possessing a large number of

An examination of the several figures of Pls. I–III will bring to the attention some interesting and rather anomalous illustrations of another phase of the structures under consideration. As will be seen, there is here almost every degree of intergradation between the perfectly symmetrical cruciform aboral junction of the chymiferous canals and the perfectly circular canal about the base of the gastric pouch into which the radials connect before their connection with the gastric cavity. By careful injections through the radial canals I have clearly demonstrated a direct continuity of the chymiferous system throughout these several channels. Little doubt can therefore remain concerning the fundamentally similar character of these various structures. Nor is it more doubtful that in function they are fundamentally similar; and while concerning the question of their significance in relation to the affinities of the Hydro- and Scyphomedusae there may be room for wide difference of view, that they serve similar functions in both is highly probable, if not quite certain.

In passing to the consideration of a specimen of unusual form, it should be noted that in the origin of spurs, extra canals, etc., they were with very slight exceptions, which seem to me easily explained, centrifugal, *i.e.*, from the central toward the peripheral portions of the body. The apparent exceptions are shown in Pl. II, Figs. 4 and 7, where portions of canals extend from the margin toward the center. As will be noted, however, there are in both cases spurs from the central region in the line of the peripheral branches which would strongly

radial canals, many of them show bifurcations toward the margin, and in not a few cases are there found centripetally developing canals similar to those of Carmarina. This medusa likewise shows many other phases of variation, spurs, anastomoses, etc., of canals, but no details will be undertaken in this connection.

suggest that there had been complete unions of these partial canals at an earlier stage and that the present condition was the result of atrophy such as is shown in Figs. 6 and 9. It would seem therefore quite just to conclude that these several structures, spurs, partial canals, loops, etc., have had their development usually from the central pouches or canals and not from the peripheral or marginal canal.

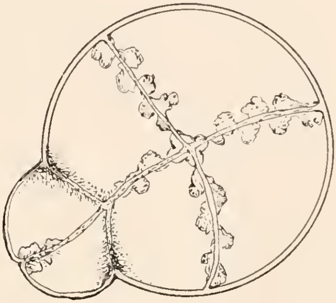
Fig. 13.

The unusual specimen, to which reference is made in this connection, is shown in Fig. 13 of the text. It would appear to partake somewhat of the nature of a monstrosity and in some respects of the nature of a marginal bud, suggestive of a secondary medusa. Aside from the general form there is little to confirm this possibility; there is no sign of manubrium; and the canals and tentacles are quite continuous with those of the primary medusa. As will be noted, there are vestiges of gonads upon the peripheral termination of the median canal, while the branches are wholly devoid of any signs of such structures. Only a single specimen of this character was found and it exhibits another aspect of erratic variation.

Gonads.

In the comparisons of gonads only specimens apparently sexually mature were taken (as noted before, no distinction was made between sexes). In the cases wholly devoid of gonads the size and other organic conditions were considered as sufficient to warrant the conclusion that they were probably of such age and general development as are usually correlated with perfect sexual maturity. In the whole number of specimens examined 3.6 per cent showed numerical variation of the gonads; of specimens with less than the normal number, two per cent; of those with more than the normal, 1.2 per cent; of specimens without trace of gonads, .4 per cent. As will be seen in comparing these ratios with those concerning the radial canals, there is here again a slight tendency toward the smaller or trimerous forms, though not specially marked, especially when account is taken of the fact that only on specimens with more than the normal canals would additional gonads be found, while it was not rare to find in tetramerous forms specimens with only three gonads, or even less, one tetramerous specimen having a single gonad. Concerning variations of the several gonads of individual specimens no account was taken, owing to the difficulty of determining relative differences in organs loosely suspended in sinuous folds, as are these in Gonionemus, and by the further fact of the continued growth and successive discharge of the sexual products, as seems to be the case here.

Manubrium.

As in most medusae, the manubrium is a rather prominent and important organ. In correlation with the tetramerous organization of the medusa, the manubrium, including in this general term the basal gastric pouch and oral opening and lobes, is of similar form and adjustment. As will be noted, however, by a comparison of the several tables, there are many exceptions, or, in other words, considerable variation. In most cases, however, as comparison will show, there is in the variation an obvious correlation with other variations, notably with that involving the radial canals. But here again the exceptions are sufficiently numerous to warrant the conclusion that there is in this organ itself individual variation, apparently devoid of any adaptive end or relation.

Aside from the facts of meristic nature above noted, there are features of variation which would seem to be of a purely substantive character. For example, in several specimens the manubrium was greatly extended lengthwise, reaching in some cases quite beyond the velum, occasionally as much as one-fourth its total length. While of course this organ is very extensile, yet in many hundreds of specimens examined alive, in many cases while the animal was engaged in engulfing food, I have never seen the manubrium extended beyond the velum.

While no emphasis is placed on this feature of variation, it is yet worthy of note in comparison with such medusae as Coryne, Dipurena, etc., in which the greatly elongated manubrium feature is rather distinctive.

In Fig. 14 is shown an interesting and anomalous feature which is more or less monstrous, namely, a spike-like growth from one side of the basal portion of the manubrium. While in some respects it might be comparable with an oral tentacle of Margelis or Nemopsis, still only in a somewhat remote way. It was rather rigid, yet devoid of any chitinous or other rigid support. As will be seen, it has the form of an elongate, attenuate process, about twice the length of the manubrium. It would seem, as suggested above, to be a wholly unique if not anomalous structure, without evident correlation with, or adaptation to, any other organ or function.

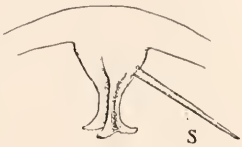

Fig. 14. — Semidiagrammatic sketch of manubrium of medusa showing anomalous projecting spur, *S*.

Tentacles.

As compared with Eucope, Obelia, Podocoryne, and many other genera, there seems to be a very different order and relationship among the tentacles of Gonionemus. In the smallest specimen measured the diameter was but 2 mm., and the number of tentacles twenty-nine. The largest specimen found measured 19 mm., and the number of tentacles was sixty-eight. That mere size is not, however, determinant of numbers will be seen when it is stated that a specimen measuring 4 mm. had thirty-nine tentacles, while one measuring 6 mm. had but thirty. While, as stated above, the largest measured specimen had sixty-eight tentacles, two others measuring 15 and 16 mm., respectively, had each seventy-two tentacles, and a specimen measuring 14 mm. had seventy-one tentacles. While it should be stated that these observations were made upon specimens preserved in formaldehyde, which may have thereby suffered some shrinkage, still since the preservative was in all cases the same medium and of the same, or very nearly the same, per cent, they would presumably be similarly affected.

However, the matter is not in any wise dependent upon data of this character. Even a glance at Figs. 9–12 will show, though diagrammatically, the relative number and distribution of the tentacles about the margin, while an inspection of the tables will show how very variable is this matter.

Bifurcation of tentacles, tentacular spurs, etc. — In all some fifteen specimens were found having variations involving one or more of the features indicated under this head. As noted by Agassiz and Woodworth ('96) in Eucope, the origin of spurs is usually from the base, as is also the doubling of the tentacles, as shown in Pl. IV, Figs. 2, 7, and 10. In several specimens there was an evident bifurcation of the terminal portion, as shown in Pl. IV, Figs. 5, 6, 8, and 9. In the specimens shown in Pl. IV, Fig. 6, this had occurred in close conjunction with the peculiar suctorial bulbs or pads so characteristic of this genus, while in Fig. 8 it is shown as having occurred somewhat proximal to these structures. A single specimen was found having three of these organs on a given tentacle at considerable intervals. In several specimens the tentacular pads or bulbs associated with the bases of the tentacles exhibited peculiar cordate lobings, sometimes on the outer border, more frequently on the inner edge, or from both, as if about to divide, though in no case was division found to be complete in a given bulb. However, this feature will acquire some significance in relation to the double and triple tentacles shown in Fig. 2, where the pads are correspondingly double and triple.

As compared with the specimens of Figs. 7 and 10, however, there will be seen no such correlation,—a fact which would suggest a measure of caution concerning the possible relation of the apparent division of the basal pads and the doubling of tentacles. This caution is further emphasized by the fact that in their origin new tentacles appear wholly apart from these pads, which only after some time are gradually developed on their ventral bases.

I am unable to agree with Agassiz and Woodworth (*op cit.*, p. 139) that these double and triple tentacles are due to coalescence of the bases. Whatever may be the case with Eucope,

TABLE I. — TETRAMEROUS SPECIMENS.

RADIAL CANALS.	GONADS.	GASTRIC LOBES.	ORAL LOBES.	TENTACLES.
4	4	4	4	12, 12, 12, 12.
4	4	4	4	12, 12, 15, 16.
4	4	4	4	17, 15, 14, 5.
4	4	4	4	15, 14, 14, 14.
4	4	4	4	12, 11, 13, 13.
4	3	3	3	20, 18, 10, 13.
4	4	4	4	10, 10, 11, 8.
4	4	4	4	16, 15, 17, 17.
4	4	4	4	14, 12, 12, 11.
4	4	4	4	16, 16, 16, 16.
4	4	4	4	14, 14, 14, 14.
4	4	4	4	15, 15, 15, 15.
4	4	4	4	14, 14, 14, 14.
4	4	4	4	12, 12, 12, 8.
4	4	4	4	17, 17, 21, 14.
4	4	4	4	17, 17, 19, 17.
4	4	4	4	16, 16, 16, 16.
4	4	4	4	11, 11, 11, 11.
4	4	4	3	15, 15, 16, 16.
4	4	4	4	12, 11, 12, 10.
4	4	4	4	13, 12, 12, 11.
4	4	4	4	16, 13, 15, 14.
4	4	4	4	15, 15, 15, 15.
4	3	4	4	13, 13, 10, 9.
4	4	4	4	13, 11, 12, 12.
4	4	4	4	11, 11, 11, 11.
4	4	4	4	12, 10, 11, 11.
4	4	4	4	13, 14, 14, 13.
4	4	4	4	17, 17, 17, 15.
4	4	4	4	14, 14, 14, 14.
4	4	4	4	15, 16, 14, 15.
4	4	4	4	16, 15, 16, 14.
4	3	3	3	12, 11, 8, 4.
4	4	3	3	11, 12, 11, 11.
4	4	4	4	12, 12, 13, 13.
4	4	5	5	21, 13, 13, 14.
4	4	4	4	11, 14, 11, 7.
4	4	4	4	14, 14, 13, 17.
4	3	4	4	14, 15, 14, 9.

Table II.

Radial Canals.	Gonads.	Gastric Lobes.	Oral Lobes.	Tentacles.
3	3	3	3	14, 20, 22.
3	3	3	3	14, 19, 19.
3	3	3	3	20, 21, 21.
3	3	3	3	16, 20, 22.
3	3	3	3	14, 18, 24.
3	3	3	3	15, 15, 17.
3	3	2	2	13, 17, 24.
3	3	3	3	12, 15, 18.
3	3	3	3	13, 15, 18.
3	3	3	3	12, 12, 21.
3	3	4	4	13, 14, 18.
3	3	3	3	15, 15, 16.
3	3	3	5	15, 17, 23.
*4	4	4	4	9, 13, 16.
*4	4	4	4	15, 15, 17.
5	5	4	4	11, 12, 14, 17, 18.
5	4	4	4	8, 12, 14, 15, 16.
5	5	5	5	7, 8, 9, 10, 11.
5	5	4	4	11, 9, 9, 11, 8.
5	5	3	3	4, 9, 11, 9, 15.
5	5	4	4	12, 10, 7, 8, 13.
5	5	5	5	12, 11, 11, 7, 11.
5	5	4	4	12, 10, 10, 11, 11.
5	5	4	4	11, 7, 9, 9, 11.
5	5	5	5	8, 9, 7, 9, 8.
5	5	4	4	6, 10, 14, 12, 10.
5	5	5	5	11, 13, 9, 10, 11.
5	5	5	5	8, 9, 15, 17, 16.
5	5	4	4	11, 10, 13, 13, 13.
6	6	5	5	6, 5, 6, 6, 6, 6.
6	6	4	4	7, 9, 8, 12, 18, 9.
6	6	6	5	7, 9, 8, 6, 4, 8.
6	6	6	5	7, 11, 7, 8, 9, 5.
6	6	5	5	11, 4, 9, 7, 7, 12.

* While in these specimens four canals were present, two in each were so closely approximated as to divide the bell into trimerous sectors.

with Gonionemus all the facts would seem to point to their independent origin, in many cases the tentacles being of conspicuously different sizes, and in others exhibiting all phases of intermediate conditions between the simple bifurcation of the terminal portion through the budding of a smaller tentacle from the side of the larger, on to the symmetrical double tentacles as shown in Figs. 3, 4, and 10.

In only one case was a trifid tentacle found. This is shown in Pl. IV, Fig. 9. However, while a trifid structure there seems to be a degeneration of the median lobe, which was in all probability the terminal portion of what was originally a normal tentacle, from which later were budded the two lateral shoots, each in turn becoming more prominent than the median tip and developing in the appropriate places the characteristic suctorial pads. The degenerating middle tip would very naturally suggest the probability that an injury might have been the predisposing cause of the secondary tips; on the other hand, it must not be overlooked that in each of the other specimens with double tips no such cause seems at all evident. I am inclined to consider the cases as simply the expression of the intrinsic capabilities of variation, more or less evident in the several classes of organs already considered.

As yet no reference has been made to the order in which second, third, and subsequent series of tentacles arise. Gonionemus, unlike Eucope, seems to have no such association of tentacle and sensory bulb as serves to locate in part the primary set of tentacles and the order of appearance of the subsequent series. While usually there is a single primary tentacle at the terminus of each radial canal, this is not invariably the case. An inspection of Figs. 9–12 will show that there may be a very wide range of variation in this respect. The following data, taken at random from many observations, will further illustrate the same general fact.

TABLE III.

Showing the number and order of succession of tentacles of three series, counted from a primary set at some arbitrary point, as a radial canal. The large figure gives the number of any given series occurring together, while the small exponent figure indicates the series concerned, *viz.*, primary, secondary, or tertiary.

A. $2^1, 1^2, 1^1, 1^3, 1^1, 1^2. 3^1. 1^3, 4^1, 2^3, 2^1, 1^3, 1^1, 1^3, 4^1, 1^2, 6^1, 1^2, 1^1. 1^2, 2^1, 1^2, 4^1, 1^3. 4^1.$

B. $2^1, 1^3. 1^2, 4^1. 1^3, 4^1, 1^2, 8^1, 1^3, 1^2, 1^3, 2^1. 1^3. 1^2, 4^1, 1^3, 2^1.$

C. $1^1, 1^2, 1^3, 2^1, 1^2, 1^1, 1^2, 1^1. 2^2. 1^3, 1^1, 2^3, 1^1, 1^2, 2^1, 1^2, 1^1, 1^2, 1^1, 1^3, 1^1, 1^2, 1^3. 2^1, 1^2, 3^1, 1^2, 1^1.$

D. $3^1, 1^2, 1^1, 1^3, 3^1, 1^2, 1^1, 1^3. 5^1, 1^2. 3^1, 1^2. 1^1, 1^3, 5^1, 1^2, 3^1, 1^2, 1^1, 1^3.$

E. $4^1, 1^3, 1^1, 1^2, 1^3, 1^1. 1^2. 3^1. 1^2, 3^1, 1^3, 3^1, 1^3, 2^1, 1^2, 1^1. 1^3, 2^1, 1^3, 3^1, 1^3, 2^1, 1^2, 1^1, 1^2. 1^1. 1^3, 1^1, 1^2, 1^1, 1^2.$

F. $1^1, 1^2, 1^1, 1^2, 1^1, 1^3, 1^1. 1^2, 1^1, 1^3, 1^1. 1^2. 1^1, 1^3, 1^2, 1^1, 1^3, 1^1, 1^3, 2^1, 1^3, 1^1, 1^3, 5^1, 1^3, 1^1, 1^2, 1^1, 1^3, 2^1. 1^2, 1^1, 1^2. 1^1, 1^3, 1^1, 1^2, 1^1, 1^3.$

G. $1^1, 1^2, 1^1, 1^2, 1^1, 1^3, 1^2, 1^1, 1^3, 1^1, 1^2, 1^1. 1^3, 2^1, 1^2, 1^1, 1^3, 2^1, 1^3, 1^1, 1^2, 1^1, 1^3, 2^1, 1^2, 1^1, 1^3, 2^1, 1^3, 1^1, 1^2, 1^1, 1^2, 2^1. 1^3, 3^1, 1^3, 2^1, 1^3, 2^1, 1^3.$

H. $1^1, 1^3, 2^1, 1^2, 2^1, 1^2, 1^1, 1^3, 2^1, 1^2, 1^1, 1^3, 1^1, 1^2, 1^1, 1^3, 2^1, 1^2, 1^1, 1^3, 2^1, 1^2, 1^1, 1^2, 1^1, 1^3, 1^1, 1^3, 1^1, 1^2, 1^1, 1^3, 3^1, 1^2, 1^1, 1^3, 1^1, 1^2, 1^1, 1^2, 1^1, 1^3, 2^1, 1^2, 2^1, 1^3, 1^1, 1^3. 2^1. 1^2. 1^1, 1^3.$

I. $3^1, 1^2, 2^1, 1^3, 1^1, 1^3, 2^1, 1^2, 1^1, 1^3. 2^1, 1^2, 1^1, 1^2, 2^1. 1^3, 1^1, 1^3, 1^1, 1^3, 1^1, 1^3, 1^1, 1^3, 4^1, 1^3, 3^1, 1^2, 1^1, 1^3, 1^1. 1^2, 2^1, 1^3, 6^1, 1^3. 2^1. 1^2, 1^1, 1^3, 1^1, 1^3.$

SUMMARY.

	PRIMARY.	SECONDARY.	TERTIARY.	TOTAL.
A.	35	6	7	48
B.	26	4	6	36
C.	18	11	6	35
D.	26	6	4	36
E.	29	8	8	45
F.	25	9	11	45
G.	29	9	12	50
H.	36	13	13	62
I.	39	7	14	60

As will be seen from these series of tabulated relations in the appearance of tentacles, there is apparently no order whatsoever. Compared with the Figs., *op cit.*, where is shown similar relation (not to say absence of relation), there can hardly be discerned any such thing as definite series or sets of tentacles arising in definite succession. On the contrary, new tentacles seem to arise wherever and whenever in the growth of the bell the adequate marginal space becomes available. And the fact that this seems so variable would appear to warrant the inference that growth occurs at irregular intervals and areas over the body of the medusa. Might not this fact throw some light upon the marked unsymmetry of such forms as those shown in Figs. 10 and 11? This suggestion would seem to find some further support in the fact that very young specimens appear to be more constant in their symmetry than those more mature.

Otocysts.

In formalin specimens there is a degree of opacity induced, especially about the marginal area of the bell, which often renders difficult any satisfactory examination of these sensory bodies. Hence only a limited number of critical determinations on this point were made, but these were sufficient to show a degree of variation both in their number and arrangement quite as marked as in that of other organs.

Normally they should occur in somewhat symmetrical order between the bases of the tentacles. This, however, is rarely the case. There seems about the same variation in their occurrence and relations as in the case of the tentacles, though I was not able to discover that the latter had any determining influence upon them. In only a few cases have I been able to demonstrate the presence of more than a single otolith in a given cyst, and in no case more than two. On this point, however, the opacity above referred to, and the abundant pigment about the bases of the tentacular bulbs were material obstructions to such determinations, and suggest tentative conclusions. In matter of shape and size these organs present likewise considerable variation. Pl. IV, Fig. 1, presents the average aspect of form at *a*, while at *b* are shown forms not unusual but variant.

Summary and Review.

1. Variation among Hydromedusae is of wider extent than had been supposed.

2. Variation is much greater in some genera than in others.

3. Variation among Hydromedusae appears to be much less symmetrical and less definitely correlated than among Scyphomedusae.

4. Many phases of variation seem to be wholly devoid of any adaptive features or tendencies.

5. The ratio of variation is higher among the tentacles than among other organs, and in many species higher than in all other organs combined, — a feature which is perhaps the most conspicuous case of adaptation apparent in the entire series.

Among the earliest references to variation in Coelenterates is that of Ehrenberg (**'37**) relative to variation in Aurelia. Later, Romanes (**'74-'76**) took up the subject with much more detail, giving an extended account of the nature and extent of variation, particularly in Aurelia, in which he figures and describes many "monstrous forms of medusae" and points out interesting correlations of radial canals, gonads, tentacles, etc.

Within recent times these observations have been much extended, notably by Brown (**'94**), who distinguished more than two per cent.

Sorby (**'94**), Herdman (**'94**), and Unthank (**'94**) have each recorded many interesting facts of variation in this medusa.

In 1895 Brown still further extended his observations upon Aurelia, and in connection therewith undertook a comparison of a large number of the Ephyrae. He was able to distinguish no less than 22.6 per cent of numerical variation in tentaculocysts, a ratio very close to that earlier determined for adult Aurelia. The observations seemed to show upon the whole a tendency toward an increase in meristic characters.

Ballowitz (**'98**) records extended observations upon Aurelia, specially with reference to the gonads. While in general there was more or less correlation in the numerical variation of these organs with the actinal lobes, it was apparently less constant than had been claimed by earlier observers. The highest number noted was seven, while three was the minimal number. One specimen in particular, which he names Ephyra abnormitat, seems to be an unusual monstrosity, having a very large balloon-shaped body with a correspondingly large manubrium.

He explains it as probably due to an enormous expansion of the top of the Ephyra, thus forming the balloon-like body.

It may not be amiss in this connection to record observations of a similar character as to numerical variations upon the Aurelias of Woods Holl which quite confirm those cited.

Observations upon the Hydromedusae seem to have been heretofore quite limited. Those of Forbes (**'48**), Agassiz (**'49**), Hincks (**'68**), Romanes (**'74–'76**), Agassiz and Woodworth (**'96**), include all the more important observations which have come to my knowledge. The latter would seem to be about the only series made upon a large number of specimens with the purpose of ascertaining the extent and character of variation in a single genus.

SYRACUSE UNIVERSITY, September 1, 1900.

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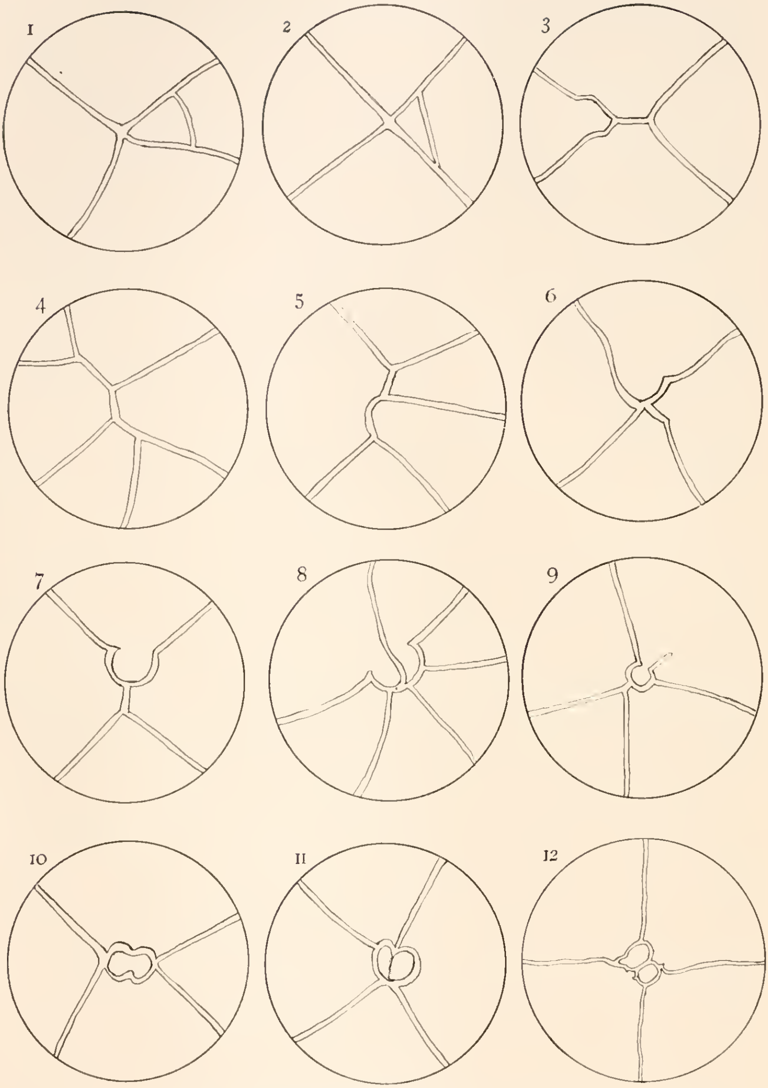
1
2
3
4
5
6
7
8
9
10
11
12

PLATE I. — Diagrammatic figures, illustrating variation in form, number, and arrangement of the radial canals.

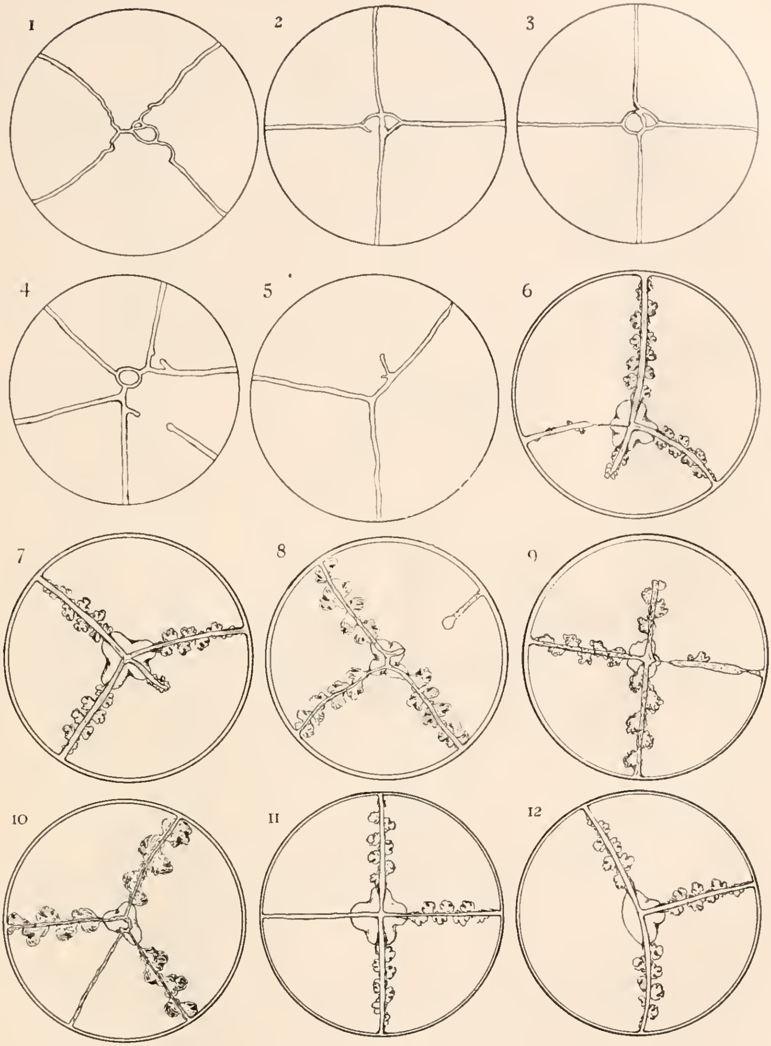
1
2
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Plate II. — Showing various phases of atrophy, spur-like branches, etc. of radial canals.

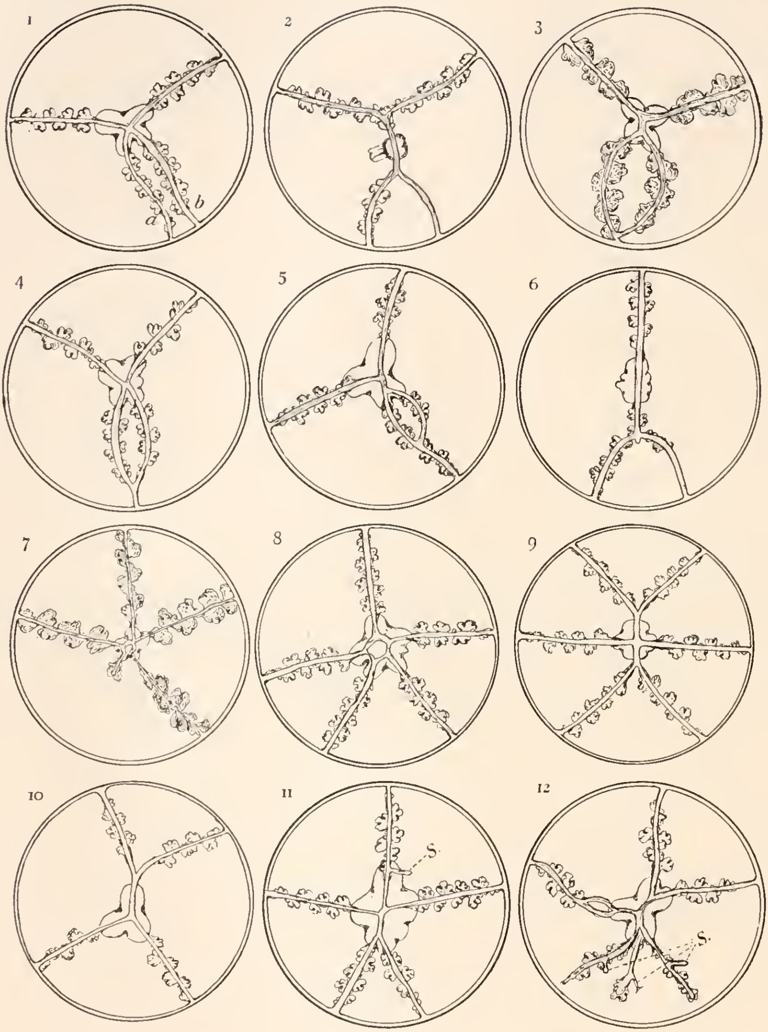
1
a
b
2
3
4
5
6
7
8
9
10
11
S.
12
S.

Plate III. — Figs. 1–6 show varying phases in the evolution of trimerism. Figs. 11 and 12 show at *S* the development of spurs.

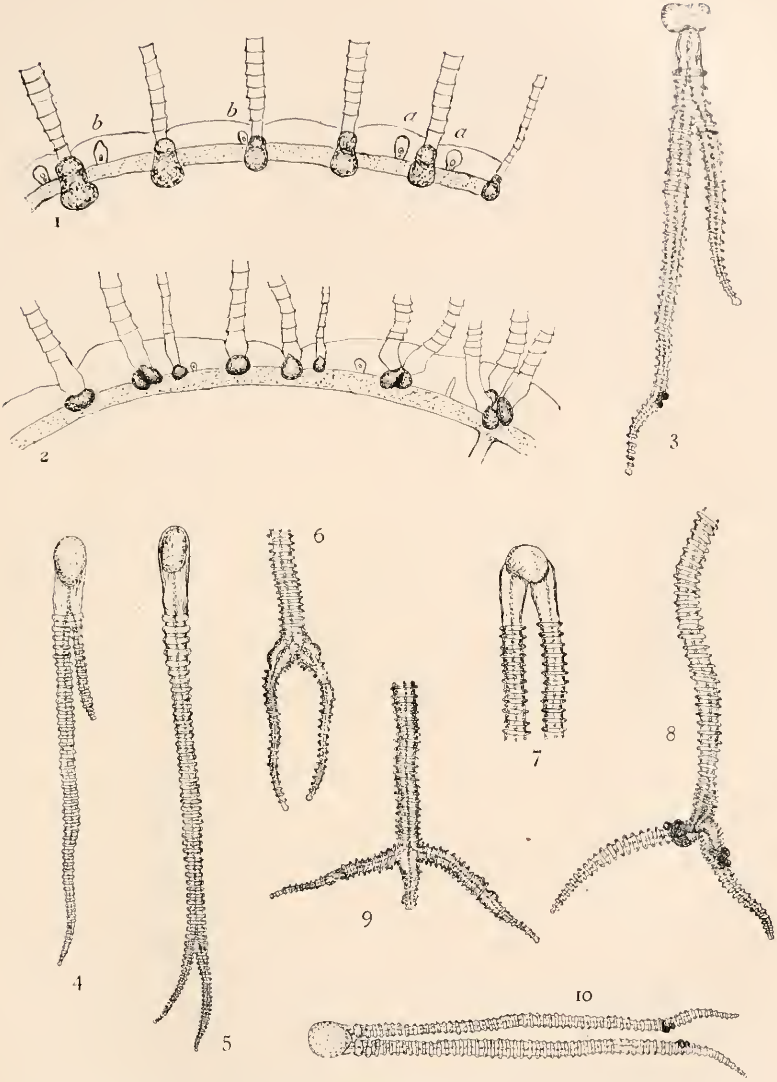

Plate IV. — Fig. 1, *a*, *a*, normal: *b*, *b*, variable forms of otocysts. Fig. 2 showing variation in tentacles. Figs. 3–10 show various phases in forking or budding of tentacles.

Volume II.] *June, 1901.* [*No. 6.*

BIOLOGICAL BULLETIN.

[From the Zoölogical Laboratory of the University of Pennsylvania.]

THE INDIVIDUALITY OF THE GERM NUCLEI

ERRATA.

In No. 5, p. 225, 19th line, read *sensory bulbs* in place of *tentaculocysts.*

On p. 228, 20th line, read *ocelli* in place of *otocysts.*

Biol. Bull.

of other animals.[1]

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[1] Rückert calls attention to the fact that partially cleft nuclei are found in the figures of various authors, particularly those of Fol (**'79**) on Toxopneustes, of Bellonci (**'84**), and of Kölliker (**'89**) on Siredon. Of course no one of these observers has interpreted these figures as showing the independence of the germ nuclei, and some of the figures referred to by Rückert probably do not show this phenomenon. For example, only one of Fol's figures (Pl. VII, Fig. 7) shows a dual nucleus, while the figure in Kölliker's text-book (Fig. 36) is probably a case of the indentation of the nuclear membrane opposite the centrosomes in the early prophase, a thing which frequently happens. Bellonci's Figs. 1 and 20 show an indentation on one side of the nucleus which may correspond to a division between the germ halves, though this must be regarded as more or less doubtful.

Volume II.] *June, 1901.* [*No. 6.*

BIOLOGICAL BULLETIN.

[From the Zoölogical Laboratory of the University of Pennsylvania.]

THE INDIVIDUALITY OF THE GERM NUCLEI DURING THE CLEAVAGE OF THE EGG OF CREPIDULA.

EDWIN G. CONKLIN.

HAECKER (**'92**) and Rückert (**'95**) have made known the interesting fact that the germ nuclei of Cyclops do not fuse but preserve their individuality throughout a considerable portion of the cleavage of the egg. Herla (**'93**) and Zoja (**'95**) have shown that the paternal and maternal chromosomes of Ascaris remain distinct at least as far as the 12-cell stage. These observations are of the greatest significance and, so far as they go, establish Boveri's hypothesis (**'91**), "that in all cells derived in the regular course of division from the fertilized egg, one-half of the chromosomes are of strictly paternal origin, the other half of maternal." So far as I am aware, similar observations have not hitherto been made in the case of other animals.[1]

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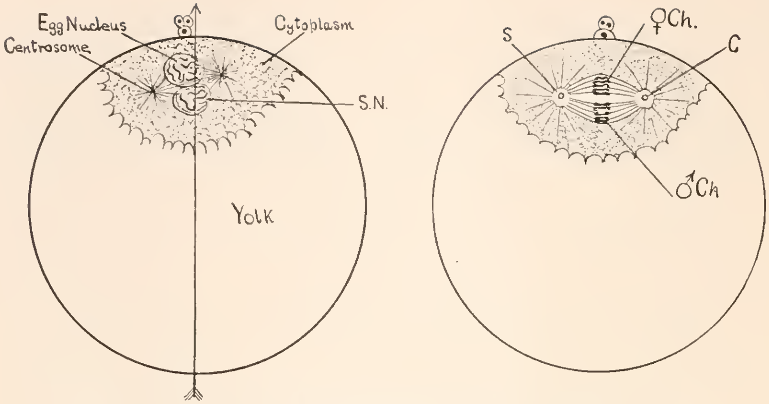
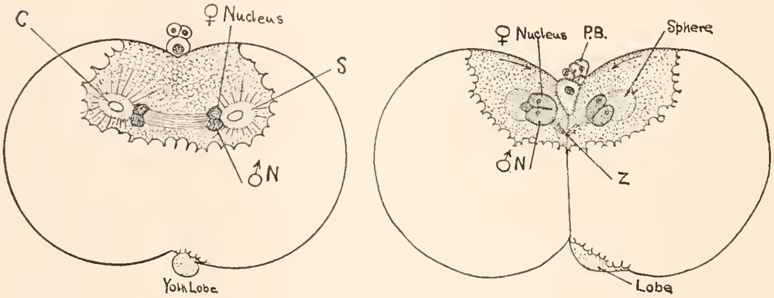
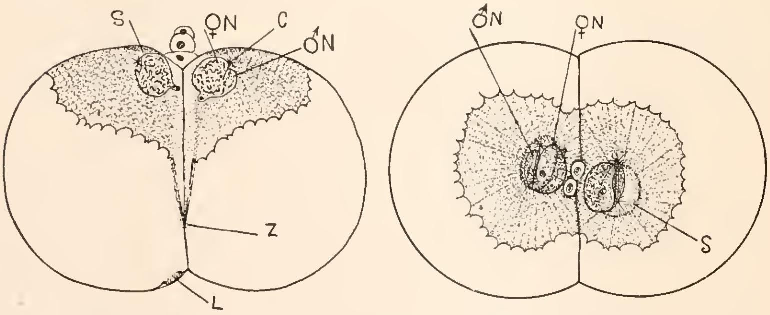
[1] Rückert calls attention to the fact that partially cleft nuclei are found in the figures of various authors, particularly those of Fol (**'79**) on Toxopneustes, of Bellonci (**'84**), and of Kölliker (**'89**) on Siredon. Of course no one of these observers has interpreted these figures as showing the independence of the germ nuclei, and some of the figures referred to by Rückert probably do not show this phenomenon. For example, only one of Fol's figures (Pl. VII, Fig. 7) shows a dual nucleus, while the figure in Kölliker's text-book (Fig. 36) is probably a case of the indentation of the nuclear membrane opposite the centrosomes in the early prophase, a thing which frequently happens. Bellonci's Figs. 1 and 20 show an indentation on one side of the nucleus which may correspond to a division between the germ halves, though this must be regarded as more or less doubtful.


Egg Nucleus
Centrosome
Cytoplasm
S.N.
Yolk
S
♀Ch.
C
♂Ch

FIGS. 1 and 2. — Prophase and Metaphase of First Cleavage. *S.N.* = Sperm Nucleus. *Ch.* = Chromosomes. *S.* = Sphere. *C.* = Centrosome.

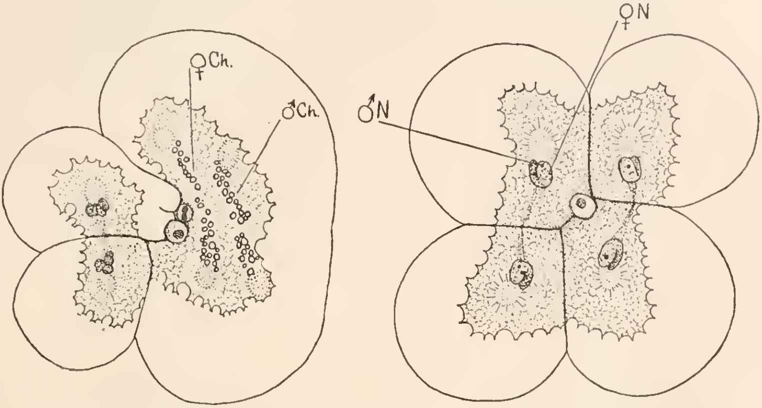

C
♀ Nucleus
S
♂N
Yolk Lobe
♀ Nucleus
P.B.
Sphere
♂N
Z
Lobe

FIGS. 3 and 4. — Anaphase and Telophase of First Cleavage, showing dual nuclei, centrosomes (*C*) and spheres (*S*), " Zwischenkörper " (*Z*), bending of spindle axis, and progressive absorption of yolk lobe.

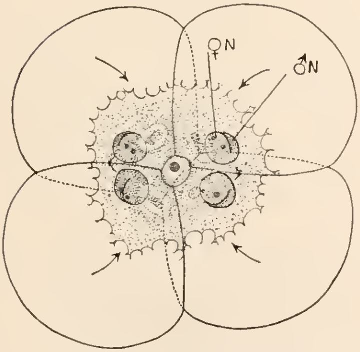


FIGS. 5 and 6. — Prophase of Second Cleavage, showing dual nuclei with central spindle lying in groove between the halves; Fig. 5 viewed from one side, Fig. 6 from animal pole. The spheres (*S*) lie over the nuclei and immediately under the cell wall; the spindle axis is bent on itself, and the "Zwischenkörper" (*Z*) is carried nearly to the vegetal pole; the nuclei shows processes projecting toward the "Zwischenkörper," and the yolk lobe (*L*) is almost completely absorbed.

In Crepidula plana I have observed a separateness of the germ nuclei in certain stages of the nuclear cycle which is of such a character that it may lead to the discovery of similar phenomena in other animals. This separateness is most easily observed in the telophase of each division, though in some cleavage cells it may be seen in the prophase also, or even throughout the resting period. At the time when the daughter-nuclei are being formed the chromosomal vesicles fuse into two



Figs. 7 and 8. — Anaphase and Telophase of Second Cleavage. Fig. 7, an abnormal egg in which the left half has divided normally and the chromosomal vesicles of the daughter-nuclei are fusing; in the right half the division figure is double with four spheres and two groups of chromosomal vesicles which have not fused; there are thirty chromosomal vesicles in each group. Fig. 8, a normal egg showing the egg and sperm constituents in each of the daughter-nuclei.



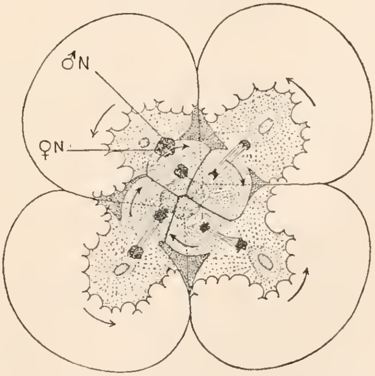


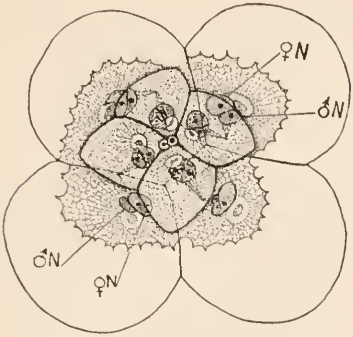
Fig. 9. — Telophase of Second Cleavage; centrosomes, spheres, and nuclei rotating in direction of arrows.

Fig. 10. — Anaphase of Third Cleavage. Egg and sperm constituents of nuclei indicated.

groups which are closely pressed together but are still separated by a partition wall (Figs. 4, 8, *et seq.*), as Rückert has shown to be the case in Cyclops. Gradually this partition wall disappears, being preserved longest on that side of the nucleus nearest the centrosome (Fig. 5). Here a groove is formed on one side of the nucleus which marks the line of contact between the two halves. In some cleavage cells this groove is visible throughout most of the resting period (Figs. 4, 9); in others it disappears during the greater part of the resting period, though it may reappear in the following prophase (Figs. 5, 6); in all cases, however, the partition wall and groove reappear in the next succeeding telophase, when it is formed again in the manner described above. I have observed the double character of the nucleus in the telophase of every cleavage up to the 29-cell stage (Figs. 1–16), and in several of the later cleavages up to the 60-cell stage, and I have no doubt that it is found in all the later cleavages, though it becomes more difficult to see as the nuclei grow smaller. While the halves of these double nuclei occupy similar positions relative to each other at corresponding stages in any cell generation, they occupy different positions at different stages and in different generations; consequently the position of the groove or partition wall which separates the halves of the double nuclei can be satisfactorily studied only in preparations of entire eggs, which may be observed from all sides. All the figures which illustrate this paper are therefore of entire eggs, though many isolated cases of double nuclei have been observed and studied in actual sections.

On each side of the partition wall which divides these double nuclei there is usually a single small nucleolus; these two nucleoli persist long after the disappearance of the partition and frequently throughout the whole of the resting period. In most if not all of the early cleavages there are two, and only two, nucleoli present in the telophase (Figs. 3, 4); but if this is succeeded by a very long resting period the number may increase to more than two, or all may fuse into a single enormously large one.

It still remains to show that these double nuclei really



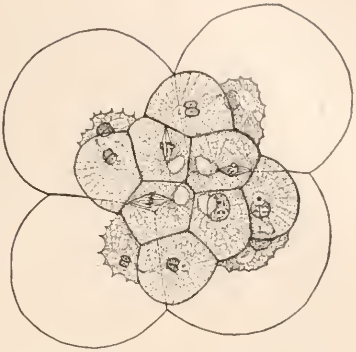
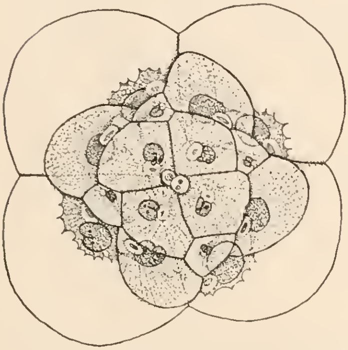


Fig. 11. — Telophase of Third Cleavage. Egg and sperm constituents of nuclei indicated; also bending of spindle axis and rotation of centrosomes and nuclei.

Fig. 12. — Telophase of Fourth Cleavage and Prophase of division of First Quartette cells. The nuclei in the telophase are dual, though from this stage on the egg and sperm constituents cannot be identified with certainty.



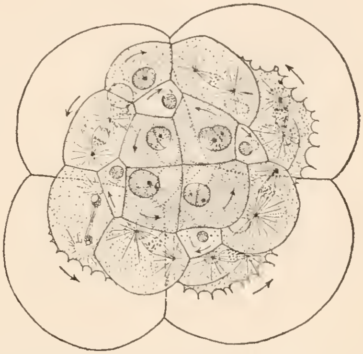
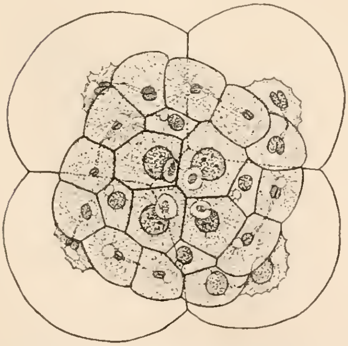


Fig. 13. — Telophase of Division of First Quartette. Dual nuclei in each daughter-cell.

Fig. 14. — Subdivision of Second Quartette and formation of Third. Dual character of nuclei shown in apical cells.



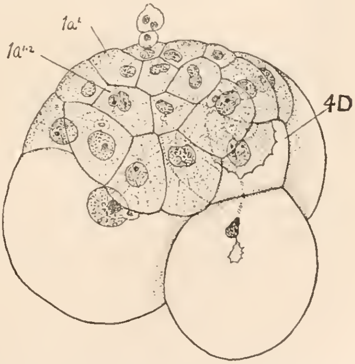


Fig. 15. — Telophase of Division of Second Quartette and of formation of Third; dual nuclei shown in almost all of these cells.

Fig. 16. — Telophase of formation of the Mesentoblast Cell (4 D) and of the second division of the First Quartette ($1\,a^1$, $1\,a^{1\cdot 2}$, etc.); dual nuclei shown in both cases.

represent the egg and sperm nuclei which have not yet lost their individuality. This cannot be demonstrated in Crepidula, for the reason that this double character is not apparent at every stage in the nuclear cycle, but it is extremely probable, as the following observations will show :

1. In the first cleavage the germ nuclei do not fuse but remain distinct throughout the prophase, and even in the metaphase they are represented by separate groups of chromosomes (Figs. 1, 2) ; in the early anaphase these groups of chromosomes can no longer be distinguished, though I think they must still remain separate, for the nuclei are clearly double in the immediately following late anaphase and telophase (Figs. 3, 4). The position of the partition wall in these double nuclei corresponds to the plane of contact between the germ nuclei ; the egg nucleus always lies more or less above the sperm nucleus, and in the telophase of the first cleavage one-half of each double nucleus overlaps the other half to a greater or less extent (Figs. 1–4). It is probable that the upper half represents the egg nucleus, and the lower half the sperm nucleus, and in all the later cleavages it is probable that the half of the nucleus which lies nearest the animal pole is from the egg, and the other half from the sperm.

2. The groove which is found on one side of the nucleus in the telophase of the first cleavage (Fig. 4) persists well into the resting stage, and a corresponding groove is found in the same position in the prophase of the second cleavage (Figs. 5, 6). The central spindle for the second cleavage lies in this groove (Fig. 6), and thus the amphiaster actually lies in the only plane in which it would be possible to halve the two parts of the double nuclei. This very fact shows that each half of a double nucleus is represented in the daughter-nuclei, and it strongly suggests that the two parts of the daughter-nuclei are derived directly from the corresponding parts of the mother-nucleus (*cf.* Figs. 6, 8). The fact that the central spindle lies in the groove separating the halves of the nucleus has been observed in the first, second, third, and fourth cleavages, and is undoubtedly a general phenomenon. There is no reasonable ground for doubting that the two parts of every double nucleus are

derived from corresponding parts of a mother-nucleus, and so on back to the egg and sperm nuclei in the first cleavage.

Since the descendants of the germ nuclei are halved at every division, it follows that successive divisions of the double nuclei cannot be at right angles to one another, since this would lead to an unequal division of the halves, or even to a division along the plane of contact between the halves. Such an unequal division might be prevented in cleavages which successively alternate in direction by the rotation of the nucleus during the resting period, or by the rotation of the spindle in the early stages of mitosis.[1]. As a matter of fact both of these methods occur in Crepidula. The nucleus usually rotates during the rest through 90°, so that although successive nuclear spindles are at right angles to one another the axis of every spindle lies in the same nuclear axis (*cf.* Figs. 3, 4, 8, 9); but in some cases the nuclear spindle does not lie in its definitive position when first formed but undergoes extensive rotation after its formation. While it is not susceptible of absolute proof, since the partition wall is absent during the later stages of the rest, it is highly probable that the plane in which all nuclear spindles lie is the plane of contact between the two halves of every nucleus.

3. In certain abnormal cleavages the double nuclei are really two entirely separate nuclei lying side by side within a single cell. Such binucleated cells may occasionally be found with the nuclei in the height of the rest, though they are more usual in the telophase or early resting period. There is usually but a single sphere and centrosome in such cells, though in one case of pathological mitosis which I have seen (Fig. 7) there are two mitotic figures side by side; the chromosomes which have reached the stage of the chromosomal vesicles have not aggregated at the poles of these spindles, but are scattered along their whole length. There are thirty of these

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[1] Rückert finds that the nuclei rotate in Cyclops even after the spheres have reached their definitive positions at the poles of the spindle; I have never observed in Crepidula a rotation of the nuclei, independent of the spindles, at so late a stage in the cell cycle.

chromosomes in each spindle, the same number that is found in each germ nucleus.

4. In each of the germ nuclei, before they come into contact, there is a single nucleolus; these nucleoli disappear in the prophase of the first cleavage, but in the succeeding telophase a single nucleolus generally appears in each half of each daughter-nucleus. The same is true of the succeeding cleavages, so that each nucleus throughout the cleavage usually has two nucleoli in the telophase or early resting stage, though the number may vary in the later resting period, as pointed out above. The fact that there is a single nucleolus in each germ nucleus, and that there is usually a single nucleolus in each half of the double nuclei of the cleavage, may possibly indicate that these halves are each derived from one of the germ nuclei. Since the nucleoli as such do not persist throughout the mitosis, may it not be possible that there is some achromatic structure in connection with them which does persist and form the basis for the new nucleoli which appear in the daughter-nuclei?

These facts make it very probable that the germ nuclei of Crepidula preserve their individuality throughout the cleavage, though their separateness may be apparent only or chiefly in a single stage of the nuclear cycle, *viz.*, the telophase. Further, it is possible, even in an advanced stage of the cleavage, to determine with considerable probability which part of a double nucleus is derived from the egg and which from the sperm, the egg half always lying nearer the animal pole than the sperm half. Finally the initial position of the mitotic spindle seems to be determined by the relative positions of the halves of the double nuclei, since the spindles when they first appear lie in the plane of contact between the two halves; the final position of the spindle and the direction of division are determined by the movements of the cytoplasm.

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THE EARLY DEVELOPMENT OF THE HYPOPHYSIS IN CHELONIA.

CHARLES ZELENY.

The following observations on the early development of the hypophysis in Chelonia are offered at this time because they throw positive light upon the derivation of the organ concerning which we at present have several conflicting views. The paper is based upon sections of Aspidonectes spinifer Ag., Chelydra serpentina L., and Chrysemys marginata Ag., which show that in these forms the hypophysis is undoubtedly of epiblastic origin. Incidentally some points regarding the relation of the preoral gut to the notochord and the head cavities will be noted, but this subject will receive fuller treatment in a subsequent paper. For the sake of convenience the subject matter will be considered under the following heads :

1. A general outline of the literature dealing with the early development of the hypophysis among vertebrates.
2. Material and methods of preparation.
3. Description of stages.
4. Summary and conclusion.

Literature.

It would be out of place in a paper such as the present to give any detailed account of the views which have been held regarding the subject under discussion. A bare mention of a few of the upholders of each of the principal views, giving the group upon which work was done, must suffice.

1. Those who have claimed a hypoblastic origin for the hypophysis are Luschka ('69), Mammalia ; W. Müller ('71), Vertebrata in general ; Dohrn ('82), Teleostii, later extended to Vertebrata in general ; Hoffmann ('88), Lacerta ; Prather ('99), Amia.

2. Those who have claimed an epiblastic origin for the hypophysis are Mihalkovics (**'77**), Aves and Mammalia ; Balfour (**'78**), Elasmobranchii ; Orr (**'87**), Lacerta ; Lundsborg (**'94**), Salmonidae ; Dean (**'96**), Amia ; Haller (**'96**), Vertebrata in general ; Hoffmann (**'96**), Elasmobranchii ; Melchers (**'99**), Lacertilia. Of these Orr (**'87**), although he describes the hypophysis as of epiblastic origin and so figures it in his sections, nevertheless considers it probable that hypoblast cells may take some part in its development.

3. Those who have claimed that the hypophysis is partly of epiblastic and partly of hypoblastic origin are Kupffer (**'93**), Acipenser and Ammocoetes ; Valenti (**'95**), Amphibia (Bufo) ; Nussbaum (**'96**), Mammalia ; and considered probable by Orr (**'87**), Lacerta.

It is of special interest to note that even this partial list gives each of the three views a large number of the groups of vertebrates upon which to base its general character.

Material and Methods.

The material upon which the following observations are based was obtained in Minnesota during the summers of 1898 and 1899. The embryos of Aspidonectes are from Grey Cloud Island in the Mississippi River below St. Paul, and those of Chrysemys and Chelydra are from the neighborhood of Hutchinson. The fixing fluid used was Gilson's mercuro-nitric mixture. The embryos were stained *in toto* in haema-calcium or in paracarmine. The series of sagittal sections were found to be the most helpful in determining the cell-layer from which the hypophysis is derived, and the following descriptions are taken entirely from such sections.

Description of Stages.

The following stages will be figured and described :

Stage A. The hypophysial evagination has not yet appeared.

Stage B. The hypophysial evagination is very evident and the pharyngeal membrane has not yet been broken.

Stage C. Slightly older than Stage B. The pharyngeal membrane has been broken.

Stage D. The downward growth and enlargement of the fore-brain have pushed the hypophysis back from its primary relation to the broken ends of the pharyngeal membrane.

Stage E. The hypophysis has been shifted backward so as to assume a position relatively far back in the pharyngeal cavity.

Stage F. The hypophysis has become differentiated into a terminal, broad, sac-like part and a narrower connecting stalk.

Stage A.

In an embryo with five or six mesoblastic somites and in which the medullary folds have not yet united above to enclose a medullary canal the hypophysial evagination has not begun to form. A median sagittal section of such an embryo, however, shows the relation of the parts surrounding the point at which the hypophysis will appear at a later stage. Fig. 1, Pl. I, represents a diagrammatic median sagittal section of an embryo of Chrysemys marginata 2.5 mm. in length and in which there are five distinct mesoblastic somites with indistinct traces of a sixth. The figure is a combination of the six sections nearest to the median line. Although the medullary groove is still open above, the medullary folds in the head region have grown to a considerable height, as represented by the dotted line *D.* At the same time the whole anterior region has been folded and bent downward. The fold of the blastoderm which comes up over the head as a result is the proamnion (*Pa.*), and consists of epiblast and hypoblast. At the bottom of the head fold the hypoblast has traveled back much farther than the epiblast, leaving a space in which the cardiac mesoblast (*Cm.*) develops. As we trace this hypoblast (*End.*1) forward along the floor of the fore-gut (*F.G.*) we find that it is bent ventrally so as to come into contact with the epiblast. This point of contact, represented by the double-headed arrow (↕), is the region at which the mouth will be formed. In front of this we may recognize a short, wide, preoral gut (*Pr.G.*). This is a true preoral gut and not an apparent one caused by the downward bending of the head region ; for we must consider the sharp angle in the hypoblast at *Pr.G.* in the figure, and not the part back of it where the mouth will appear, to have been the original extreme anterior end. Thus we may consider all the hypoblast below and posterior to this angle to belong to the ventral wall of the alimentary canal, and all that above and posterior to belong to the dorsal wall. Following the dorsal wall we find that it immediately divides into two parts, one of which is the notochord (*Nc.*) and the other the dorsal wall of the gut (*End.*$_2$). For this reason the hypoblast forming the ventral wall of the alimentary canal, and continued out at the ends of the embryo into the flat outlying blastodermic region, may be called the "primary hypoblast," and the hypoblast of the dorsal wall the "secondary hypoblast." Rex ('97), in his work on the duck, and Davidoff ('99), in the embryo of Platydactylus, have found similar relations of the notochord to the hypoblast. The terms "primary hypoblast" and "secondary hypoblast," which are used above, are taken from the paper of the latter author. It is important to note in this connection that my sections show a distinct line of demarcation between the epiblast and the hypoblast in this region, so that the mass of cells surrounding the preoral gut is distinctly hypoblastic and not a mass of undifferentiated cells. The arrow with a feathered shaft (➷) shown in Fig. 1 in the epiblast directly under this point marks the position and direction of the future hypophysial pocket. The very plain line of division between the epiblast and hypoblast excludes the possibility that any of the hypoblast cells may take part in this ingrowth of the epiblast.

Stage B.

In an embryo 5.2 mm. in length and with twenty-one mesoblastic somites, such as is shown in median sagittal section in Pl. I, Fig. 2, and Pl. II, Fig. 3, the brain has developed with great rapidity. The cephalic flexure having proceeded at the same time, the region around the preoral gut is greatly compressed. The cavity of the preoral gut itself has become very small and its dorsal wall is doubled back on itself. The notochord also at its anterior end near the point where it joins the hypoblast has become very much twisted and curved, giving it a knotted appearance in sagittal section. However, the parts are easily recognized as having the same relation as those in Stage A (Fig. 1). In Stage B (Fig. 2), as before, the dorsal wall of the fore-gut is the "secondary hypoblast" and the ventral wall the "primary hypoblast," the division line between the two being the point where the notochord joins the hypoblast. The pharyngeal membrane is still intact but breaks up very soon after this stage. Directly in front of the place where the mouth opening will appear the epiblast bends sharply back on itself to follow the brain, forming a pocket in which the cells are taller than in the neighboring parts of the epiblast. This is the beginning of the outpocketing which will eventually form the hypophysis. The evaginating layer of cells is clearly distinct from the hypoblast cells of the preoral gut, as is well shown in Pl. II, Fig. 3, which gives an enlarged view of the hypophysial region of Fig. 2. Starting from the condition of the stomodeal epiblast in the earlier stage as shown in Fig. 1, we see that an epiblastic groove was originally formed at the point marked by the feathered arrow (↖) just in front of the future mouth by the forward and downward bending of the fore-brain. It is at the bottom and in the middle of this groove that the thickening and outpocketing of the cells start, and later form the epiblastic pouch which becomes the oral portion of the hypophysis.

Stage C.

In an embryo but slightly older than the one last described the pharyngeal membrane has already been broken. Fig. 4, Pl. III, represents a diagram of a median sagittal section of such an embryo. On account of a lateral bend in the neck region it was not possible to obtain a section which would show the connection of the notochord and the preoral gut at the same time with the mouth opening and the hypophysial evagination. The canal (*H.C.*) between the two premandibular head cavities is, however, shown, and the small mass of cells connected with it and directed toward the hypoblast of the fore-gut is the strand which connects the head cavities with the preoral gut. The preoral gut itself is not shown in this figure, the cavity (*F.G.'*) being a part of the fore-gut which has assumed a position anterior to the mouth because of the bending of the alimentary canal, which takes place at the same time with the cephalic flexure. Except for the break in the pharyngeal membrane the relation of the hypophysis to the epiblast is the same as in Stage B. The hypophysial outpocketing is here, as before, on the epiblastic side of the mouth opening and is undoubtedly made up entirely of epiblast cells.

Stage D.

In Fig. 5, Pl. III, we have a median sagittal section of a somewhat later stage. The points *p* and *p'* show the position of the ends of the broken pharyngeal membrane, and the dotted line (*P.M.*) between them represents the former position of the now ruptured membrane. The hypophysial pouch (*Hyp.*) is shown very distinctly on the epiblastic side of the membrane. It has, however, been pushed back from its original position with relation to the point *p'* by the rapid growth of the fore-brain. The anterior end of the notochord (*N.*) is still more curved and wrinkled than in the last stage, but it retains its connection with the anterior wall of the preoral gut (*P.G.*) by means of a string of cells. In this string of cells we see the section of the canal (*H.C.*) which connects the two anterior or premandibular head cavities. The hypophysial evagination from the very beginning is in close contact with the wall of the infundibulum, but the two layers of cells always remain clearly distinct. The epiblast cells of the hypophysis also remain clearly distinct from the hypoblast cells of the preoral gut.

Stage E.

Fig. 6, Pl. IV, is a diagram of a section of Chelydra serpentina. It shows a continuation of the same process of enlargement of the fore-brain and the consequent pushing back of the hypophysial evagination, so that the latter finally appears to lie far back in the pharyngeal cavity. However, here, as before, the dotted line *pp'* shows the original position of the now broken membrane. At this stage the notochord has severed its connection with the fore-gut, but it is still joined to the mass of cells which connects the two head cavities. These cells still surround a canal, so that there is a passageway from the premandibular cavity on one side of the head to the corresponding cavity on the other side.

Stage F.

Fig. 7, Pl. IV, represents a median sagittal section of Aspidonectes spinifer, and Figs. 8 and 9, Pl. V, represent sections of the same series, respectively, three and six sections to the side of the median line. In these it is seen that there is no evidence of the canal which connected the two head cavities, and the notochord shows no sign of the former union with the hypoblast. The hypophysis has begun to constrict in the basal region and to enlarge in the terminal region so as to show a division into a narrower basal stalk and a wider terminal sac-like portion.

At a considerably later stage than the above the infundibulum sends out a pouch-like evagination, which from the beginning is in close contact with the wall of the oral sac and forms the infundibular part of the hypophysis.

Summary and Conclusion.

The foregoing series of sections furnish a clear chain of evidence in favor of the epiblastic origin of the hypophysis in Chelonia. Stage B (Figs. 2 and 3) itself is a conclusive proof of such an origin. Here with the pharyngeal membrane yet unbroken we find that the evagination is on the epiblastic side of the membrane. The distinct limiting line which marks the inner border of the hypophysial pouch excludes the supposition that the hypoblast cells may take a part in its formation. Even at the early stage represented in Fig. 1 there is a distinct limiting line between the epiblast and hypoblast at the point where the hypophysis will later appear.

After the mouth opening has appeared (Stage C, Fig. 4) we find the hypophysis at first in the same relative position as in Stage B. Then the great increase in size of the fore-brain forces the epiblastic pocket to a position far back in the pharyngeal cavity, so that Stages D and E when considered alone would lead one to believe that the hypophysis is of hypoblastic origin in this group. That such is not the case is made evident by following the whole series of changes through all the different stages. There can be no doubt that in the Chelonia at least the oral evagination which goes to form the hypophysis is of epiblastic origin. As regards the infundibular portion there is no essential difference of opinion and its development need not be touched on here.

The bearing of the above conclusions on the paleostome theory of Kupffer and the neostome theory of Dohrn is of some interest. According to Kupffer, the hypophysis was originally a canal connecting the fore-gut with the epiblast, and represents an ancestral œsophagus which came up in front of the fore-brain and was replaced by the modern œsophagus at the time when the mouth was forced to a more ventral position by the enlargement of the brain. Dohrn, on the other hand, has picked out the epiphysis and hypophysis as remnants of the old annelid œsophagus which went up through the brain. Both of the above views presuppose some connection of the hypophysis with the hypoblast. The sections of turtle embryos which are described in the present paper give no evidence of such a connection at any stage.

In conclusion I wish to express my sincere thanks to Prof. H. F. Nachtrieb, who suggested to me the investigation of chelonian development and has aided me in many ways during the progress of the work.

DEPARTMENT OF ANIMAL BIOLOGY,
UNIVERSITY OF MINNESOTA, December, 1900.

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ABBREVIATIONS USED IN CONNECTION WITH THE FIGURES.

Ao.	Aortic arch.
Cm.	Cardiac mesoderm.
Ect.	Epiblast.
End.$_1$	"Primary hypoblast."
End.$_2$	"Secondary hypoblast."
Ep.	Epiphysis.
F. B.	Fore-brain.
F. G.	Fore-gut.
H. B.	Hind-brain.
H. C.	Canal connecting the two premandibular head cavities.
Ht.	Heart.
Hyp.	Hypophysis.
Hyp. S.	Stalk of hypophysis.
I.	Infundibulum.
M.	Mouth opening.
M. B.	Mid brain.
Md.	Floor of medullary groove.
Nc. N.	Notochord.
Pa.	Proamnion.
P. G.	Preoral gut.
P. M.	Pharyngeal membrane.
. . .	Dotted line above *Md.* in Fig. 1 represents the height to which the medullary folds have risen.
↖	Position and direction of the hypophysial evagination.
↕	Pharyngeal membrane and position of future mouth opening.

PLATE I.

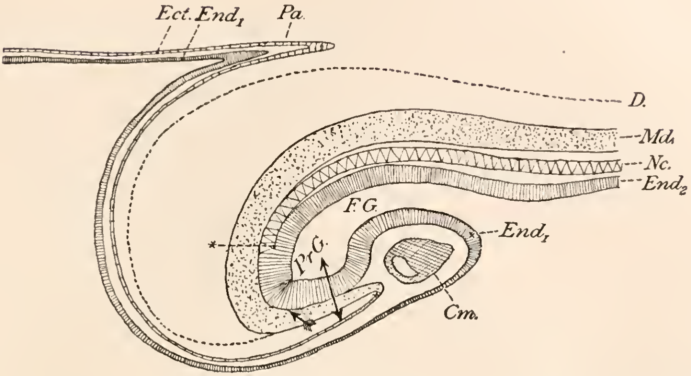
Ect.
End_1
Pa.
D.
Md.
Nc.
End_2
F.G.
End_1
PrG.
Cm.

FIG. 1 (Pl. I), Stage A. — Combination diagram formed from the six sections nearest to the median sagittal section. Embryo of Chrysemys marginata. Mesoblastic somites = 5½. Lg. of embryo = 2.5 mm. × 62.

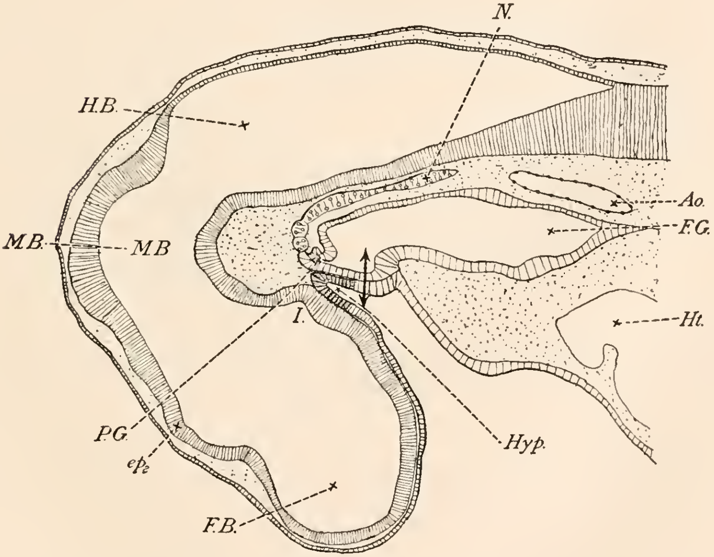
N.
H.B.
M.B.
MB
Ao.
F.G.
I.
Ht.
P.G.
ep_2
Hyp.
F.B.

FIG. 2 (Pl. I), Stage B. — Diagram of the median sagittal section of an embryo of Aspidonectes spinifer. Lg. = 5.2 mm. Mesoblastic somites = 21. × 50.

PLATE II.

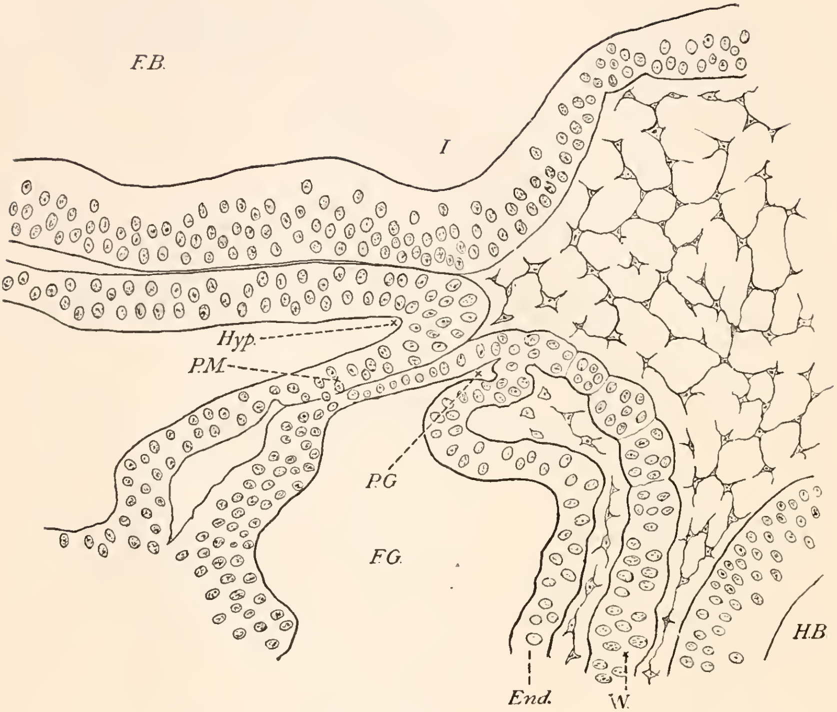
F.B.
I
Hyp.
P.M.
P.G
F.G.
H.B.
End.
W.

Fig. 3 (Pl. II), Stage B. — Median sagittal section of Aspidonectes spinifer. Part of Fig. 2, but with Magn. × 200.

PLATE III.

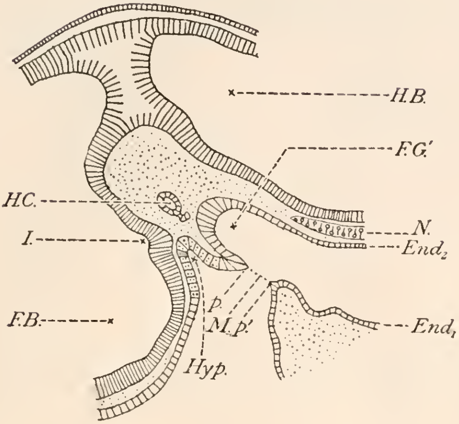
H.B.
F.G.'
H.C.
I.
N.
End$_2$
F.B.
p.
M.p.'
Hyp.
End$_1$

Fig. 4 (Pl. III), Stage C. — Median sagittal section of A. spinifer
Lg. = 5.7 mm. Mesoblastic somites = 21. × 50.

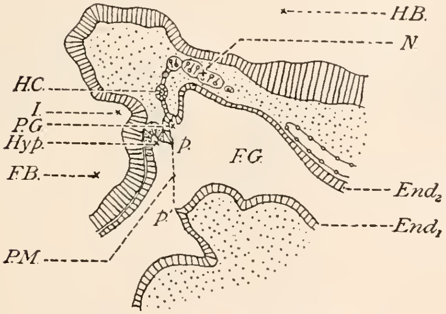
H.B.
N
H.C.
I.
P.G.
Hyp.
p.
F.G.
F.B.
End$_2$
p.'
End$_1$
P.M.

Fig. 5 (Pl. III), Stage D. — Median sagittal section of A. spinifer.
Lg. = 6.0 mm. × 50.

PLATE IV.

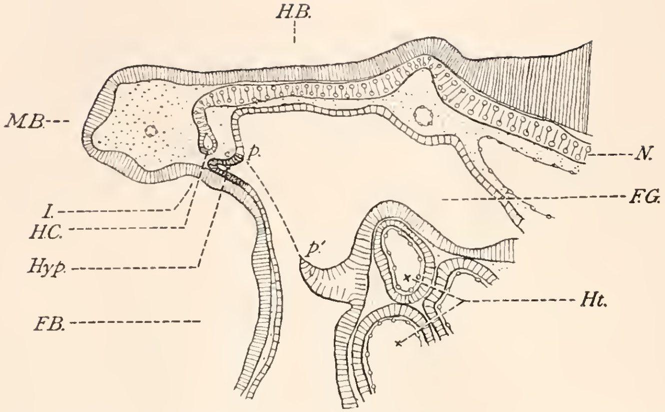
H.B.
M.B.
p.
N.
F.G.
I.
H.C.
p'.
Hyp.
Ht.
F.B.

FIG. 6 (Pl. IV), Stage E. — Median sagittal section of Chelydra serpentina. Lg. = 7 mm. × 50.

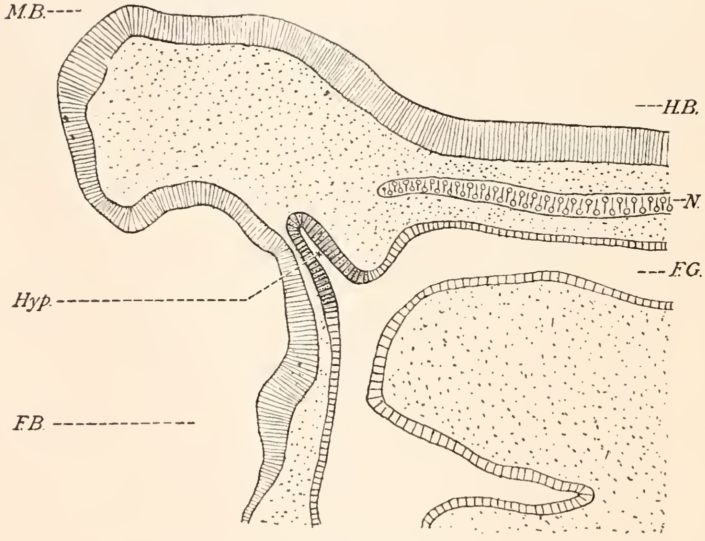
M.B.
H.B.
N.
F.G.
Hyp.
F.B.

FIG. 7 (Pl. IV), Stage F. — Median sagittal section of A. spinifer. Lg. = 7.5 mm. × 50.

PLATE V.

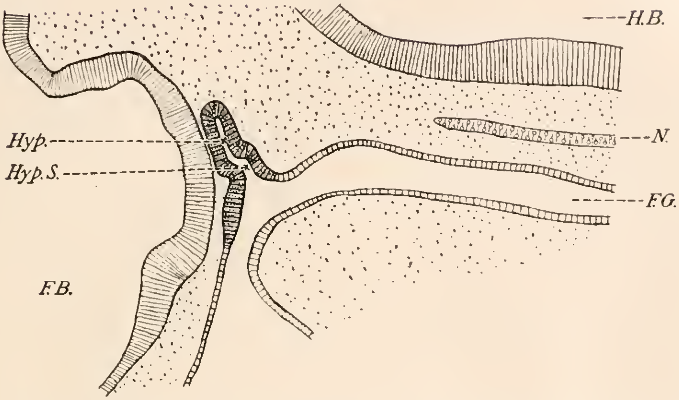
H.B.
Hyp.
Hyp. S.
N.
F.G.
F.B.

FIG. 8 (Pl. V), Stage F. — Sagittal section from the same series as Fig. 7, but three sections (40 μ) to the side of the median line. × 50.

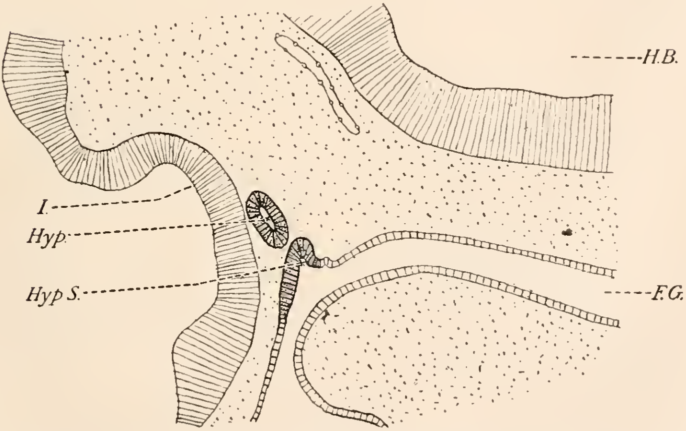
H.B.
I.
Hyp.
Hyp S.
F.G.

FIG. 9 (Pl. V), Stage F. — Sagittal section from the same series as Fig. 7, but six sections (80 μ) to the side of the median line. × 50.

ON PHORONIS PACIFICA, *SP. NOV.*

HARRY BEAL TORREY.

DURING the past summer eight specimens of *Phoronis* came into my hands. Five were collected in June, 1894, in Humboldt Bay, California, by an expedition from the University of California. Three were brought back from Puget Sound by the Columbia University expedition of 1897. As the occurrence of *Phoronis* on the Pacific coast has never been recorded, and it is eminently desirable that all localities in which this interesting form may be obtained should be made known to naturalists, I have undertaken to describe this material, which represents a single species hitherto unknown.

The following table will indicate the distribution and date of first description of all the known species of *Phoronis*. For the species presently to be described I propose the name *P. pacifica*.

P. hippocrepia Str. Wright.[1]	1856	Great Britain.
P. ovalis " "	1856.	
P. (*Crepina*) *gracilis* Van Ben.[2]	1858.	
P. Buskii McIntosh.[3]	1881	Philippines.
P. australis Haswell.[4]	1882	Port Jackson, N.S.W.
P. Kowalevskii (Caldwell)[5] Benham.[6]	1883	Naples.
P. psammophila Cori.[7]	1889	Messina. Port Jackson?
P. Sabatieri Roule.[8]	1889	Gulf of Lyons.
P. architecta Andrews.[9]	1890	North Carolina.
P. ijimai Oka.[10]	1897	Japan.
P. pacifica.	1901	Humboldt Bay, California; Puget Sound, Washington.

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[1] *Proc. Roy. Phys. Soc. Edin.*, vol. i (1856), p. 165; *Edin. New Phil. Journ.*, vol. iv (1856), p. 313.

[2] *Ann. Sci. Nat.*, 4th ser., vol. x (1858), pp. 11–23.

[3] *Proc. Roy. Soc. Edin.*, vol. xi (1881), p. 211; *Chall. Rep. Zoöl.*, vol. xxvii (1888), 27 pp.

[4] *Proc. Linn. Soc. N. S. W.*, vol. vii (1882), pp. 606, 607.

[5] *Proc. Roy. Soc. Lond.*, vol. xxxiv (1883), pp. 371–383.

[6] *Quart. Journ. Micr. Sci.*, vol. xxx (July, 1889), pp. 125–158.

[7] *Dissert. Prag* (Juli, 1889); *Zeitschr. f. wiss. Zool.*, vol. li (1890), pp. 480–568.

[8] *Compt. Rend. Acad. Sci. Paris*, vol. cix (1889), pp. 195, 196.

[9] *Ann. Mag. Nat. Hist.*, 6th ser., vol. v (1890), pp. 445–449.

[10] *Annot. Zool. Japon.*, vol. i (1897), pp. 147, 148.

Cori has discussed in an interesting fashion all save the last four species. Roule's *P. Sabatieri* and Andrews' *P. architecta* were apparently unknown to him, though descriptions of both were published before the date of his manuscript. *P. Sabatieri* is known to me only through a meager description. It differs from the other European forms in size and habit, and approaches *P. architecta* in these respects. The latter possesses the simple lophophore and comparatively small number of tentacles (60) of the European species. It may be distinguished from them (with the exception of *P. Sabatieri*, with which it may prove identical) by its larger size, its straight tubes and solitary habit, its strong longitudinal muscles (excepting *P. psammophila*), the presence of a ciliated groove in the digestive tract, and possibly by a separation of the sexes. While it agrees fairly well with *P. Buskii* in size, it differs from that species in the other characters enumerated, as well as in the complexity of the lophophore and the number of tentacles. It is thus more closely allied to the European than to the Australian and Philippine forms.

The differences between *P. australis* and *P. Buskii* are merely of habit and size, which has caused Benham to suggest their identity on the supposition that these differences are due to dissimilar environmental conditions.

The description of the Japanese species has been inaccessible, so that I can state nothing with regard to it save its existence.

It is an interesting fact that no one has cared to segregate the species of *Phoronis* under more than one generic name, and indicates the trifling character of the differences which serve to distinguish them. We may separate them, however, into two groups widely separated geographically. In the one belong the European forms, including, perhaps, the American *P. architecta.* In the other belong *P. australis* and *P. Buskii.* We may disregard the Japanese species on account of dearth of information, and the *P. psammophila* which Haswell has found at Port Jackson and which may have been brought from the Mediterranean on a ship's bottom.

P. pacifica occupies a place intermediate between these two groups both geographically and anatomically, but is somewhat more closely related to the American than to the Australian species. In size it resembles *P. Buskii*, as well as in the complexity of the lophophore; though instead of three coils in the

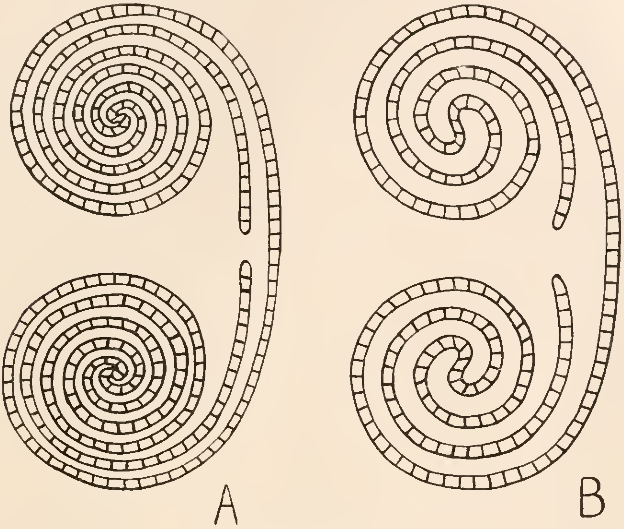

Fig. 1. — Diagrammatic cross-sections of the lophophores of *P. australis* or *P. Buskii* (*A*), *P. pacifica* (*B*).

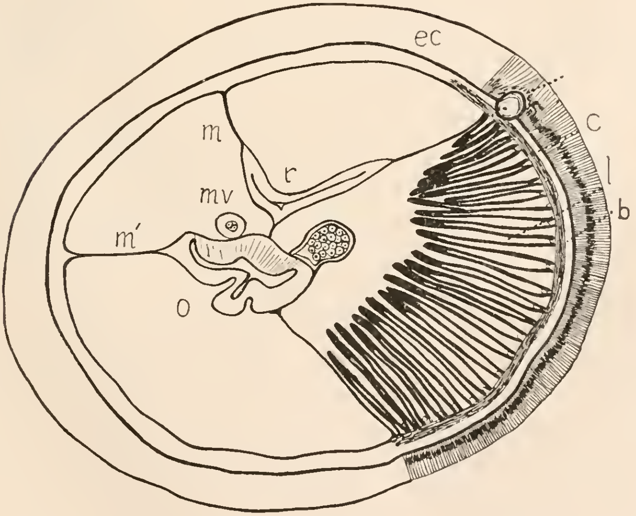

Fig. 2. — Section through the upper third of *P. pacifica;* but one quadrant detailed; semi-diagrammatic. *b*, basement membrane; *c*, circular muscle; *ec*, ectoderm; *l*, longitudinal muscles; *m*, *m′*, mesenteries; *mv*, median vessel; *n*, nerve; *o*, oesophagus; *r*, rectum.

spirals of the lophophore there are one and one-half to two, and a correspondingly smaller number of tentacles (170–200), Fig. 1, *A*, *B*. In the strength of its longitudinal muscles (Figs. 2, 3) it departs from *P. Buskii* and even surpasses *P. architecta*, resembling the latter species in the possession of a specialized ridge in the digestive tract (though this does not pass into the groove that Andrews describes), in the structure of the nervous system, lophophore organ and tube, in habit, and in the possible separation of the sexes.

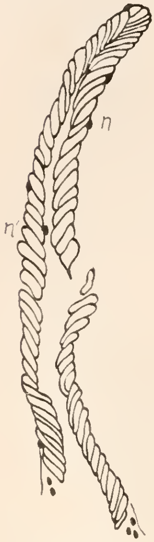

Fig. 3. — Cross-section of one longitudinal muscle ridge of *P. pacifica*, slightly broken.

It is not my intention to enter into a full and detailed anatomical description of *Phoronis*, which the labors of Benham and Cori have rendered largely unnecessary. But a few words on some points may not be out of place.

In one of the Puget Sound specimens spermatogonia and spermatocytes were found packed around the blood vessels in the aboral regions of the body, but no spermatozoa nor ova. The aboral ends of all the Humboldt specimens were wanting, so it was impossible to determine whether they were monoecious or dioecious. In one nephridium three ova were found unaccompanied by spermatozoa; the first polar body was forming in one. As there was no sign of spermatozoa in these eggs, it is probable that in this species fertilization takes place either externally or within the nephridium. It is quite possible that the sexes may be separate, or ova and sperm may be produced by the same individual but not simultaneously.

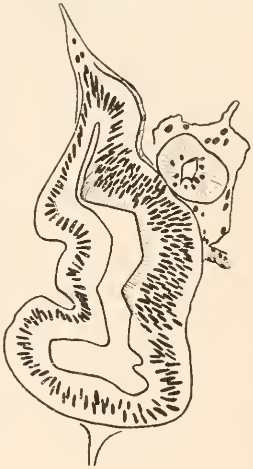

Fig. 4. — Cross-section of the oesophagus of *P. pacifica*, showing the ridge, its base in contact with the median longitudinal blood vessel.

The blood corpuscles have a conspicuous yellow color and measure 10–15 μ in diameter.

The ciliated ridge was present for a considerable distance in the oesophagus, but could not be seen in the stomach either as a ridge or a groove. In Figs. 2, 4 it is indicated in section, where it appears to be a shallow groove, an appearance probably due to the folding of the wall of the oesophagus. Its position relative to the longitudinal blood vessels is identical with that described for *P. architecta*. The nuclei stain more intensely with haematoxylin than the other nuclei of the oesophagus, and are crowded together usually in several layers. These facts make the area quite conspicuous in section.

The muscles reach their greatest development in the oral third of the body, where they form more than eighty high narrow ridges. In the aboral third these are reduced to a very inconspicuous layer, though still retaining their identity, being separated throughout their length by characteristic folds of peritoneal epithelium.

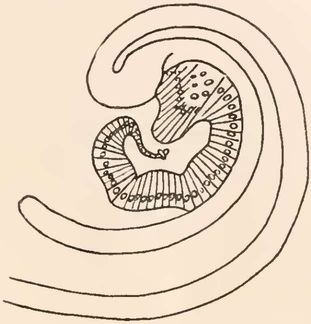

Fig. 5.—Semi-diagrammatic cross-section of lophophore organ within the concavity of the lophophore.

There is a delicate peritoneum covering the muscle ridges, the nuclei only (Fig. 3, *n*) being seen with ordinary powers of magnification. Occasionally a similar nucleus is found within the fold of muscle (*n'*).

The nervous system is constructed as in *P. architecta*, with one interesting exception. The two longitudinal cords, which are of exceedingly unequal length, instead of ending in the nerve ring of the lophophore, are continuous across the median line at the level of the median mass of ganglion cells. The loop thus formed is closely applied posteriorly to the latter and touches the lophophore nerve on each side of the rectum, apparently without fusing at either point. Just how intimate this contact is cannot be determined from my poorly fixed material. The brevity of the descriptions of this portion of the nervous system in *P. architecta* and other species leads me to suspect that the seemingly exceptional condition in *P. pacifica* may prove to be of more general occurrence.

The lophophore organ is extremely variable and may be present or absent, as in *P. psammophila.* It may resemble that of *P. australis,* though differing somewhat in shape (Fig. 5). In this case it is simple, with a thickened glandular epithelium lying for the most part against the inner circle of tentacles, and an outer free non-glandular edge of much lower cells. In another case, however, it had the form of the same organ in *P. architecta* and *P. psammophila,* as described and figured by Andrews and Cori, being composed of a basal lobe and a distal "carpel-like organ." This condition seems to have been attained by the addition of the "carpel-like organ" to the structure (basal lobe) which corresponds to the entire organ in *P. australis.*

The following is a diagnosis of the species, from material preserved in alcohol and formalin :

Total length may be 9 cm., of which the tentacles represent from 2½ to 4 mm.

Diameter, 1½ to 2 mm.

Lophophore spirally coiled, each spiral possessing from 1½ to 2 complete turns.

Tentacles 170 to 200.

Lophophore organ present or absent; extremely variable in form.

Each animal occurs singly and completely fills tube.

Tube straight, cylindrical, composed of delicate chitin, encrusted with fine sand grains.

Ridge of thickened epithelium in the descending limb of the digestive canal, just beneath the median longitudinal blood vessel.

Longitudinal nerve trunks unite across median line between mouth and anus.

Longitudinal muscles in numerous very high and narrow folds which reach their maximum in the distal third of the body.

Sexes possibly separate.

Localities: Puget Sound, Washington; Humboldt Bay, California, on sand and mud flats that may be uncovered by the tide.

Columbia University, January, 1901.

ON MUSCLE REGENERATION IN THE LIMBS OF PLETHEDON.

ELIZABETH W. TOWLE.

SPALLANZANI (1768) and Bonnet (1777) showed that a salamander whose limbs have been cut off has the power to regenerate new ones. This discovery has been confirmed by later writers, and although some histological work has been done, yet the method of regeneration of the muscle bundles has not been worked out. There are several possibilities: first, the old fibers might break down at the cut ends and the new ones develop from the indifferent tissue so formed, each old muscle thus completing itself independently. Or, the cut muscles might degenerate along their entire length, and new ones take their place; or some of the old muscles might degenerate, new ones being formed from this tissue, while some fibers might break up into smaller new fibers. An attempt has been made in this work, not so much to follow the origin of the cells in detail as to discover the general processes taking place in the leg that lead to the formation of the new muscles. The regenerating limbs of *Plethedon cinereus* were used. They were studied by means of serial sections.

In addition to this histological study, I have also experimented on a number of American urodeles in order to see in which ones regeneration of the limbs takes place. For this purpose a number of the commoner forms have been studied, and in connection with these results a statement is given of the previous observations on European forms.

I.

Method. — One of the anterior limbs of *Plethedon cinereus* was removed halfway between elbow and hand. The regenerating limbs were put up at intervals varying from four days to two weeks. They were fixed in corrosive acetic, hardened for two or three days in 95% alcohol, and then decalcified for from six to eight days in a nitric acid solution (HNO_3 sp. gr. 1.42, 2 vols. + H_2O, 98 vols.) which was changed daily. They were finally hardened again for three days in 95% alcohol, embedded and cut. Some limbs were stained *in toto* with borax carmine, but the best results were obtained by the method used by Byrnes ('**82**), *viz.*, staining on the slide in Delafield's haematoxylin, followed by a wash of picric acid in absolute alcohol. This latter method differentiates the muscle substance very clearly.

Eleven stages were preserved at the following intervals:

Time of Operation.	Time of Killing.	Age of Stump.
1. May 4, 1900.[1]	May 14, 1900.	7 days.
2. “ “ “	“ 18, “	11 “
3. “ “ “	“ 29, “	22 “
4. Oct. 22, 1899.	Nov. 20, 1899.	29 “
5. “ “ “	Dec. 5, “	44 “
6. “ “ “	“ 15, “	54 “
7. “ “ “	“ 15, “	54 “
8. “ “ “	Jan. 5, “	75 “
9. “ “ “	“ 5, “	75 “
10. “ “ “	“ 20, “	90 “
11. “ “ “	March 22, “	151 “

Transverse sections of this series were cut. Nos. 1, 2, and 3 were stained with haematoxylin and picric acid; 4, 5, and 6 with borax carmine; 7 with borax carmine and picric acid; 8 and 9 with Biondi-Ehrlich solution;[2] 10 and 11 with haematoxylin and picric acic. In addition, normal limbs were cut and stained in a similar manner and used for comparison.

Results. — For convenience in description I shall consider the sectioned limb as made up of three Regions: I, that between the cut and the elbow; II, the Region just above the cut; and III, the growing end. In the earliest stages Region III does not, of course, exist.

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[1] It will be noticed that stages 1, 2, and 3 were preserved later in the year, but observations will be described in the above order.

[2] This stain was not successful, and the stages were replaced by one stained with haematoxylin and picric acid.

In the first of the series of transverse sections changes in the cut muscles are already noticeable, the most striking being the increase in the number of nuclei, especially in the outer fibers of the limb. This increase can be seen in Region I as far up as the origin of the muscles at the elbow. In the outer fibers the muscle tissue is becoming thinner and disappearing, and while the outlines of fibers and bundles are not lost, they are much less clear than in the normal limb. The inner bundles, however, are but little affected, and extend unbroken to the cut end, which is at this time not yet entirely covered by ectoderm. No mitosis is seen in this section.

In stage 2 the changes are more marked. There has been a continued increase in the number of nuclei in Regions I and II, the outlines of the outer fibers and bundles are lost, while the muscle substance has disappeared except for disintegrating fragments here and there, contrasting sharply with the thin cytoplasm of the neighboring cells. The inner fibers still extend to the end of the limb, which is now covered entirely by several layers of ectoderm. In the neighborhood of the cut three or four mitotic figures are to be found. In the third stage Region III begins to appear as a small knob of undifferentiated tissue behind the cap of ectoderm. In this knob and for a short distance above it among the outer cells karyokinesis is not uncommon.

If we compare the following stage (4) with the normal limb, the principal changes that have taken place will be very clearly brought out. In Region I the increase in number of nuclei is very great. Even as far as the elbow two to four nuclei may be found in a section of a single fiber, often crowded together so as almost to fill it (Fig. 1). Many nuclei are also scattered between the fibers. Below the elbow the number of nuclei increases, the outlines of the outer fibers are completely lost, and the outer half of the limb, which is normally solid muscle, is seen to be made up of a dense mass of nuclei surrounded by loose protoplasmic substance, with here and there

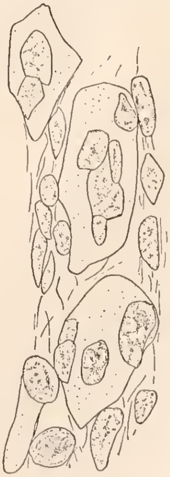

Fig. 1.

clumps of disintegrating muscle tissue. The outlines of the inner fibers are somewhat less distinct than in the preceding stage, and some of the bundles seem to be splitting up. This condition is represented in Fig. 2. Most significant is the fact that at no stage is any karyokinesis found among the muscle fibers, although the increase in the number of nuclei is enormous. Passing through Region II of this stage, the number of old fibers decreases and the scattered nuclei increase, until at the plane where the cut was made all old fibers disappear and we reach Region III, which is made up entirely of closely crowded nuclei, each surrounded by a small amount of protoplasm. Karyokinesis is first seen in II, a short distance above the cut, among the outer cells, *never in the muscle fibers*, and the number of dividing cells increases toward the growing tip until it becomes quite large.

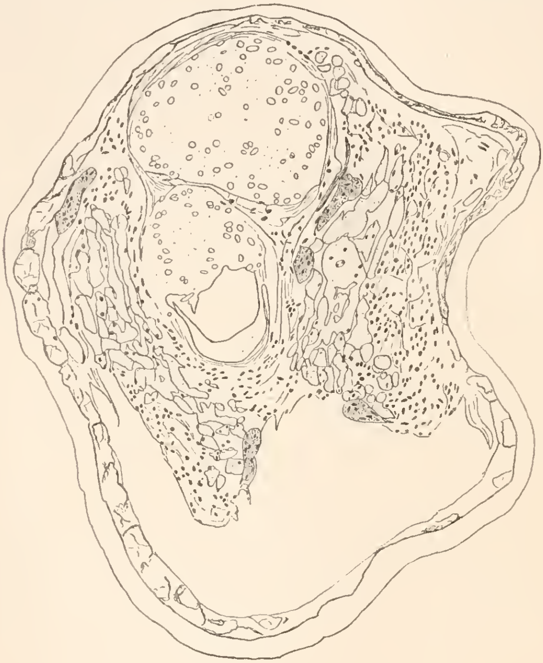

FIG. 2.

In the fifth stage a further difference is to be noted. We find in Region I, in the inner part of the limb, the old muscle fibers, often with several nuclei, and broken frequently into quite small fragments. Outside these are numerous nuclei, as before, but now surrounded by very distinct muscle tissue (Fig. 3). This tissue has formed often about several nuclei in a group, and has no distinct walls; there seem to be as yet no distinct muscle fibers. But the line of separation between the old muscle fibers and the new tissue is distinct, and a few old fibers can be traced to the region of the cut, although at that level the greatest part of the tissue is new. New muscle substance has appeared about the nuclei for a short distance below the cut; but it decreases as we pass down, until we find only crowded nuclei and thin protoplasm. In this region, as before, numerous karyokineses are seen.

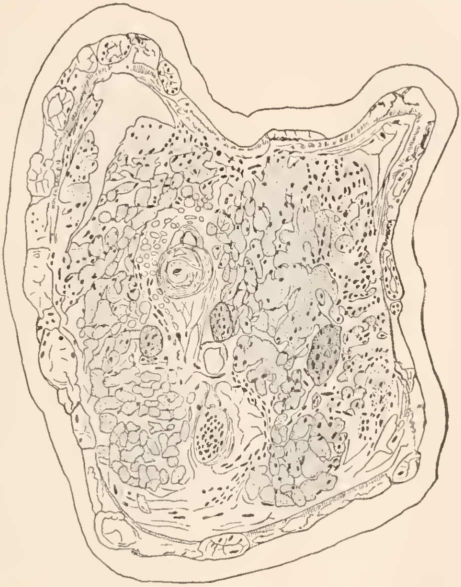

Fig. 3.

In Region I of the sixth stage all the muscle fibers are small and the definite line between the old and new is lost. The majority of the fibers contain in cross-section but one nucleus, though some may contain two or three, and in general the smallest fibers and most nuclei are on the outer side of the limb. This is especially noticeable in Region II, where the outer (new) fibers are exceedingly small. The muscle tissue decreases in amount as we pass to Region III, until it is all lost. Cells dividing by karyokinesis appear at this level.

Stage 7, though of the same age as the preceding, is somewhat further differentiated, and in this the new fibers are more rounded and have assumed a more characteristic form (Fig. 4). A comparison with the normal shows smaller fibers and great excess of nuclei, there being often two or three to one fiber and many outside the fibers.

The later stages need not be described in detail. As the limb grows longer the formation of new muscle tissue progresses farther down toward the tip, the new fibers being always small and containing several nuclei. The number of nuclei outside the fibers decreases, until in stage 11 the muscles look quite normal, and the number of nuclei is excessive only in the region of the foot, which is at this time clearly differentiated. Karyokinesis is found, I believe, without exception, near the growing end, never in the upper regions.

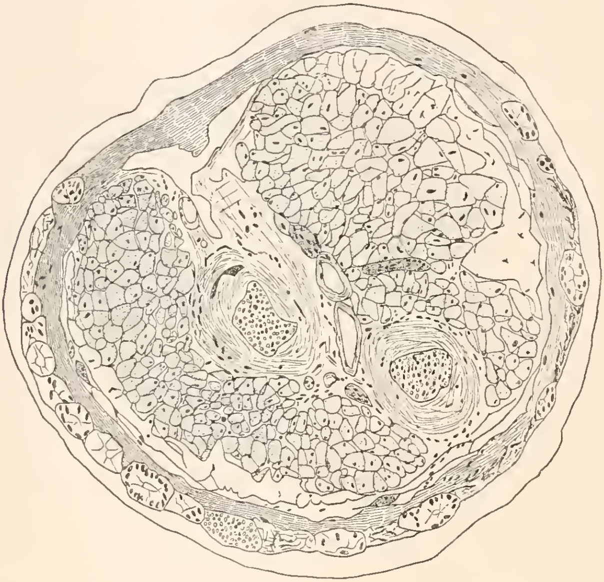

FIG. 4.

The first appearance of any definite grouping of cells appears in stage 5, where the arrangement into bundles is foreshadowed. As the fibers form, the division into bundles becomes more distinct, until in stage 11 they are all differentiated as far down as the foot, and here we can see by the arrangement of nuclei where the bundles are to be.

In the process of regeneration described above there are certain things to which I wish to direct especial attention. In the first place there is a great increase in the number of nuclei *within the old fibers*, but in no case is any karyokinesis found there. This degenerative process in the old fibers must therefore take place by direct division of the nuclei. Instances of this division are shown in Figs. 5 and 6. To this division and to the disintegration of some of the old fibers is due the enormous accumulation of nuclei in the outer part of the limb (Fig. 2). The cells so formed then begin to divide by karyokinesis in the region of the cut, and thus a further increase in their number takes place. In these outer cells new muscle tissue forms and the new fibers are built up. A certain number of the old fibers remain in the middle of the limb, and in these the muscle tissue never disintegrates, though it splits longitudinally.

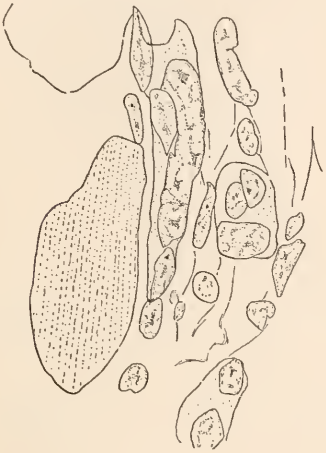

Fig. 5.

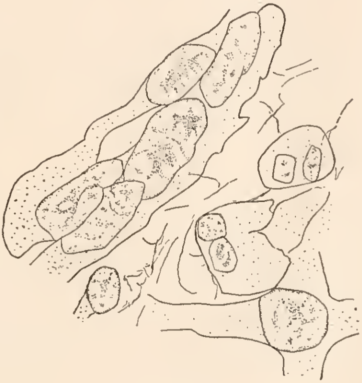

Fig. 6.

Again, as at any one level the number of nuclei far exceeds the number of fibers in a normal muscle, a great number of them must, between the early stages and the fully formed limb, either degenerate or be transported (*cf.* Figs. 3 and 4). That this is so is easily seen, for two reasons: (1) when the new fibers form, at a given level several nuclei are often included in one fiber; when the limb is full-grown there is only one; (2) among the newly formed fibers, but *between* them, are many scattered nuclei; the majority of these disappear in later stages.

One further point should be mentioned. In stage 5 the line of distinction between new and old fibers is clear, owing to their difference in size. In stage 7 this distinction has disappeared. This is due not only to an increase in the size of the new fibers, but to a decrease in the old. This decrease is, I believe, due to the longitudinal splitting of such of the old fibers as are left (Fig. 7. *a* and *b*).

Beside the stages described above, four others were preserved and cut longitudinally. This was a somewhat difficult operation, for the new part forms at an angle with the upper arm, and it is hard to orient the piece in such a way as to insure true longitudinal cutting of the muscle fibers. The material was preserved at the following intervals:

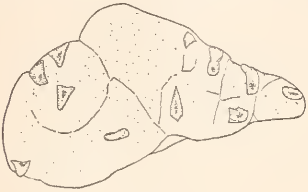

FIG. 7 *a*.

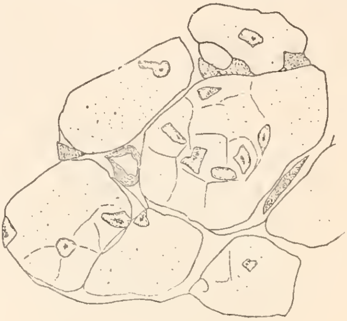

FIG. 7 *b*.

Time of Operation.	Time of Killing.	Age of Stump.
1. Jan. 29, 1900.	Feb. 2, 1900.	4 days.
2. " " "	" 12, "	14 "
3. " " "	" 19, "	21 "
4. " " "	" 28, "	30 "

The first of these shows no transformation at the cut ends of the muscles, but the ectoderm has closed in over the wound. In the second there is a considerable increase in the thickness of the ectoderm, and under it a collection of scattered nuclei, exactly similar to the tissue in the growing end of later stages. As the limb grows in length this tissue increases in amount and a number of mitoses are seen in it. Degeneration of the old fibers is distinctly noticeable in stage 3, and fibers are found, as before, filled with nuclei, but there is no karyokinesis in any muscle fiber.

SUMMARY.

The main changes that take place in the muscles of *Plethedon cinereus* during the process of regeneration are as follows:

1 (*a*) In the cut muscles the nuclei divide directly and in the outer bundles the fibers disintegrate, leaving masses of nuclei with a small amount of cytoplasm. (*b*) Some of the cells so formed later divide mitotically, and by them new muscle substance is laid down. (*c.*) As the number of nuclei is, however, far in excess of the normal number of muscle fibers, many nuclei must degenerate or be transported.

2. The fibers of the inner bundles do not disintegrate, but split longitudinally, giving rise to smaller fibers, which are soon indistinguishable from those formed as described in 1.

3. The arrangement into muscle bundles first becomes clear at the end of about six weeks.

4. There is no change in the muscles of the upper arm.

II.

The following is a brief summary of the main observations that have been made on the power of regeneration of the limbs in European species of salamanders.

Spallanzani (8), in 1768, published a number of observations on aquatic salamanders, presumably species of Triton. In these he found that any or all of the limbs will regenerate, no matter what the species, size, or age of the animal, the larger ones regenerating more slowly than the smaller forms. Regeneration will take place, he maintained, even when the limbs are disarticulated.

Bonnet, 1777 (2), confirmed Spallanzani's observations, finding that in *Triton cristatus* the hands and fingers will regenerate.

Von Siebold (7), in 1828, recorded abnormal regeneration of the fingers of *Triton cristatus*.

Higginbottom, in 1847 (4, p. 29), observed that in Triton the limbs will regenerate at a temperature of from 48° to 57° F.

Philippeaux (6), in 1866, was the first to prove conclusively that limbs when disarticulated will not regenerate. His experiments were made on *Triton cristatus* and Axolotl.

Dumeril (3), in his paper of 1867, in the course of other observations, notes the fact that in an Axolotl the two anterior limbs regenerated after injury.

Wiedersheim, 1875 (1, p. 95), found that the toes of *Triton cristatus* will regenerate, while there is no regeneration of the limbs in Proteus and *Siren lacertina.*

Erber, 1876 (4, p. 34), notes regeneration of the feet of *Siren lacertina.*

Goette, 1879 (5), records the regeneration of a leg of Proteus after a year and a half. Regeneration also occurs in Amphiuma and Siren, in *Triton cristatus, T. taeniatus*, and their larvae.

Weismann (9), in The Germ Plasm, 1893, says that the limbs of *Salamandra* regenerate, while in *Triton marmoratus* regeneration is slight or absent.

Barfurth (1), in 1894, reports regeneration of the feet and digits of *Triton taeniatus* and *Siredon pisciformis.* This regeneration is normal or abnormal according to the plane and method of the injury.

I have experimented on the following forms, removing a fore foot and a hind foot from different individuals of each species: *Plethedon cinereus, Spelerpes ruber, S. guttolineatus, Desmognathus ochrophaea, Manculus quadridigitatus, Amblystoma opacum, Diemyctylus viridescens, Amphiuma means*, and *Necturus maculatus.* Of these, all have regenerated.[1] The regeneration in Spelerpes, Desmognathus, Manculus, and Amblystoma was comparatively rapid, and new limbs were well formed in four months, though they were somewhat smaller than the old limbs. Diemyctylus was slower in reaction, while the first Necturus to show a distinct regenerated stump did so only after eight months. Other individuals of the same species showed no regeneration even at that time.

I desire to thank Professor T. H. Morgan, under whose direction this work was undertaken, for his kindly assistance during its progress.

BIOLOGICAL LABORATORY OF BRYN MAWR COLLEGE.

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[1] *Amphiuma means* was observed for only eleven weeks. At that time regeneration was slight. The regenerated stump was not sectioned.

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THE FACTORS THAT DETERMINE REGENERATION IN ANTENNULARIA.

T. H. MORGAN.

The following experiments were carried out at the Naples Zoölogical Station during June and July, 1900. As there may be no opportunity in the immediate future of completing the observations, I have determined to publish them as they stand, in the hope that the results may stimulate some one, so situated as to obtain the necessary material, to take up the questions here raised and to bring them to a more satisfactory conclusion.

Loeb's experiments on Antennularia, made in 1892, show that pieces of the stem suspended in sea water always regenerate roots at the lower end and a new stem at the upper end. The result was the same whether the apical or the basal end of the piece was uppermost, *i.e.*, whether the piece had a normal or a reversed orientation. Similar results were obtained when pieces were suspended obliquely, the high end producing always the new stem and the low the new roots, etc. These results are similar to certain results that have been obtained in plants, although Vöchting has shown conclusively in many forms that the polarity of the piece is a much stronger factor in determining the regeneration than is gravity. Loeb drew the natural inference from his results, *viz.*, that gravity determines the kind of regeneration that takes place at the ends of the piece. Driesch,[1] who examined later the regeneration of Antennularia, found that when a piece of the stem is so placed "that its basal end is freely surrounded by water," a large number of roots are formed from that end. If the end with its roots is cut off, there is generally formed from the cut end a few new roots, but also always a more or less delicate stem composed of a few tubes. This stem is negatively geotropic.

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[1] Driesch, H. Studien über das Regulationsvermögen der Organismen, I. *Roux's Archiv.* Bd. v, p. 383.

If the same end is again cut off, there develops rarely one or a few roots, but generally two or three vigorous stems. If the operation is repeated a fourth time, one or two stems are without exception produced.

There is no statement made by Driesch as to how these pieces were orientated with regard to gravity, but the results show that another factor than gravity has an influence on the regeneration. Unfortunately nothing is said with regard to what has taken place at the other end of the piece. I shall try to show that it is not improbable that this may be also a factor in the result, and if so it is possible that Driesch's results are due to this rather than to the action of the water on the free basal end, or at least both factors may be present.

My experiments were primarily undertaken in order to see how pieces would behave when fixed to a revolving wheel, but on account of the apparent disagreement between Loeb's and Driesch's results, it was first necessary to repeat the experiment of suspending pieces with two cut ends in order to see how far gravity acted upon them. In one series of experiments pieces were suspended in an aquarium by means of a silk thread. Some of these had the apical end upwards, others the basal end upwards, and still others were suspended horizontally. In nearly all cases roots developed in the course of a few days from both ends. If the ends were cut off, new roots developed again on both ends; although in one or two cases in which the apical end was uppermost a stem developed at that end. The pieces were from 3 to 5 cm. long.

By means of another device the experiment can be much more satisfactorily carried out. A small square piece was cut from a sheet of cork and a hole bored in its middle. The end of a glass rod, about 20 cm. long or longer, was pushed through the hole in the cork. If the piece of cork is neither too large nor too small, the glass rod, when put into an aquarium, will sink to the bottom until one end touches, but the other end will be held up in a vertical position owing to the buoyancy of the cork.

Pieces of the stem of Antennularia were fastened to the sides of the cork by means of two dried cactus spines, that were crossed over the stem and stuck into the cork.

In these experiments, made with pieces of different lengths, and from different parts of the old stem, the results were the same as before. In nearly every case roots developed from both ends, and even after these ends had been once removed. The experiments extended over two or three weeks. Whether, if continued longer, a stem would develop at the upper end among the roots there present, I do not know, but the results suffice to show that the most characteristic thing that occurs is the production of roots from both ends.

I was, therefore, not a little surprised to find in another series of experiments that a different result occurred. I placed in an aquarium a number of pieces of Antennularia that remained attached to the stones on which they had been found growing. Most of the pieces stood up vertically from the floor of the aquarium with the apical end upwards; a few pieces I suspended in an inverted position, *i.e.*, with the apical end downwards and the attached basal end upwards. In the former cases the apical ends did not produce roots at all, but a new stem. In the latter cases, in which the pieces were inverted, the apical end produced neither roots nor stem. Although these pieces were observed for only ten days, the time is ample to show that the pieces behave differently from pieces with two cut ends. I regret that I could not carry these experiments further.

One other result should be described, since it seems to have a direct bearing on the last experiment. In one case a very small piece had sunk to the bottom of a dish of water, where it stood with its basal end in contact with the glass. It lay there undisturbed, and attached itself at its basal end by means of new roots. *The apical end produced a shoot.* This result, taken in connection with the preceding experiment, seems to indicate that the development, or the presence of roots on the basal end, prevents the development of roots on the apical end. This result, if it prove constant, opens the way for several interesting experiments that so obviously suggest themselves as to require no further mention.

A few experiments were made with a rotating wheel constructed for the purpose. The wheel consisted of two parallel rings of wire between which, at equidistant points, were sixteen paddles (5 cm. × 8 cm.) made of oblong pieces of sheet-copper; spokes (13 cm. long) attached the rings to an axis that rotated in two sockets. When the wheel was immersed in the water of an aquarium, and a stream of water from the tap was made to play (beneath the water) on the plates, the wheel slowly revolved, making about five and one-half revolutions in a minute.[1]

Pieces of Antennularia were attached to the wheel in the following way. Sheets of cork of the same size as the copper plates were attached to the underside of the latter by wire or string. Pieces of the hydroid were fixed to the cork in different positions by means of crossed cactus spines. The pieces were, on an average, about 15 cm. from the axis of rotation. The results were entirely negative. None of the pieces produced either roots or stems; and the pieces died sooner than did those in other experiments. As this experiment was carried out in a different aquarium, I cannot be certain that the death of the pieces was not due to other causes than to the rotation. Furthermore, it is not evident from the experiment whether the rubbing of the moving ends against the water suppressed the regeneration, or whether the result is due to the continuous change of position in regard to gravity. The rotation was too slow for the action of the centrifugal force to have played any important part. Since other experiments have shown that roots may develop at both ends of a piece suspended vertically, it is improbable that in the rotating pieces the changing position in regard to the action of gravity can account for the result, and it is much more probable that the motion of the piece through the water interfered with the regeneration at the ends. The experiment needs to be repeated more often, and other check experiments carried out in the same tank.

The work that I have done on the regeneration of Antennularia, while incomplete in many ways, shows at least that other factors than gravity enter into the result. I do not question the main part of Loeb's results, for they seem to show that gravity

[1] This wheel was left at the Naples Station in the hope that it might be used by some one to continue the experiments.

is a factor in the regeneration of this form ; but the development of roots at both ends that first takes place, as I have found, but which Loeb did not observe, and the behavior of pieces attached at one end, as described in the preceding pages, show that the factors determining regeneration are more involved than previous results seem to indicate.

BRYN MAWR COLLEGE, Feb. 4, 1901.

MENDEL'S LAW OF DICHOTOMY IN HYBRIDS.

C. B. DAVENPORT.

In the study of hybrids we must, as De Vries (**1900b**) truly says, no longer pay primary attention to the degree of difference between the forms united — to whether they are species, subspecies, or varieties — but to the behavior of the peculiar characters by which the crossed individuals and their ancestors are distinguishable. For each of these somatic characters corresponds to some peculiarity of the germplasm. The behavior of the differing characters when united in the hybrid is diverse; three categories have long been recognized (Galton, **1888**, p. 12). These are: (1) *blending* heritage, illustrated by skin color in man; (2) *alternative* heritage, illustrated by human eye color; and (3) *mixed* heritage, illustrated by the piebald condition of the progeny of mice of different colors. The law of dichotomy in hybrids applies only to the second class, — alternative heritage, — although it has recently been brought forward by De Vries (**1900**) as the almost universal law of inheritance in hybrids. The law itself was first enunciated very clearly and completely by Mendel (**1865**) and deserves to bear his name. The law was, however, forgotten. It has been rediscovered independently by De Vries and by Correns (**1900**), both of whom are able to add new evidence of its validity (for *alternative* heritage!).

In his illustration of Mendel's law, De Vries first classifies hybrids into monohybrids, dihybrids, and polyhybrids, according as their parents differed in one character only, or in two characters, or in many characters. The case of inheritance in monohybrids is the simplest, and will be first considered. Mendel's and De Vries's investigations have established the following principles:

1. Of the two antagonistic peculiarities the hybrid exhibits only one; and it exhibits it completely, so as not to be

distinguishable in this regard from one of the parents. Intermediate conditions do not occur [in *alternative* heritage].

2. In the formation of the pollen and the egg cell the two antagonistic peculiarities are segregated; so that each ripe germ cell carries either one of these peculiarities.

Of the two antagonistic peculiarities united in the hybrid, that which becomes visible in the soma is called by Mendel the *dominating;* that which lies latent is called the *recessive* character. What determines which character shall be dominating is still unknown, and the determination of this point offers an enticing field of inquiry. In some cases the dominating form is the systematically *higher;* in others it is the older or ancestral form.

The law of dichotomy may now be developed. When a hybrid (monohybrid) fertilization takes place the zygote contains both the dominant quality (abbreviated d) and the recessive quality (r). In the early cleavages d and r are both passed over into both the daughter-cells; but apparently, at the time of segregation of the germ cells, the somatic cells are provided with d only, while the germ cells retain both qualities. In the ripening of these germ cells, probably in the reduction division, d and r come to reside in distinct cells, so that we have

of the female cells $50\% \, d + 50\% \, r$, and
of the male cells $50\% \, d + 50\% \, r$.

If now hybrids are crossed haphazard, a male d cell may unite with either a female d cell or with a female r cell; likewise a male r cell may unite with a female d or a female r cell. Consequently in the long run we shall have of all the zygotes

$$25\% \, d, d + 50\% \, d, r + 25\% \, r, r,$$

or 50% of the zygotes hybrid and 50% of pure blood, and of the latter half exclusively maternal and half paternal. But since the soma developed from the hybrid germ cell has the dominant character, we shall have

75% of the cases with the dominant character
25% " " " " recessive "

and this agrees with various empirical results, of which the following from Correns is instructive. A cross was obtained between a species of pea with a green (g) germ and one having a yellow (y) germ. Yellow is dominating.

Gen. 1.	31 y (hybrid) peas; produced 12 plants; these bore:			
Gen. 2.	775 y (hybrid + y) peas (= 75.8%). 21 plants were produced.			247 g (pure-blooded) peas (= 24.2%).
	7 (33%) pure-blooded y, because they bore:	14 (66%) hybrids, because they bore:		20 plants bore:
Gen. 3.	292 y peas.	462 y (hybrid + y) peas (= 76.4%).	149 g (pure-blooded) peas (= 23.6%).	670 green peas.

It is clear that if this process of crossing of the hybrids continues, the *proportion* of hybrids to the whole population will diminish; for the share of pure-blooded forms breeds true; while the originally equal share of hybrids is repeatedly halved.

If the hybrid is crossed with one of the parents instead of with another hybrid, we will

$$\text{get } (d + r)\, d = d, d + d, r; \text{ and}$$
$$(d + r)\, r = d, r + r, r.$$

In the first case all of the progeny will appear of the dominant type. In the second case one-half will appear of that type. This again agrees with experiment.

In the case of dihybrids the law of alternative heritage is somewhat more complicated. Imagine a lot of ripe germ cells with the antagonistic qualities of any pair separated according to the second principle stated at the outset. *A* indicates the one pair of qualities and *B* the other; then we shall have nine classes of zygotes, the proportion of each of which is as follows:

A. 25% *d, d* — 50% *d, r*

B. 6.25% *d,'d'*; 12.5% *d, r*; 6.25% *r, r*. — 12.5% *d, d*; 25% *d, r*; 12.5% *r, r*.

A. 25% *r, r*

B. 6.25% *d, d*; 12.5% *d, r*; 6.25% *r, r*.

Thus the first class has 6.25% purely dominant in both characters; the second class, 12.5% purely dominant in one character and hybrid in the other, and so on. Recalling that hybrid zygotes produce somas with the dominant character, it follows that the progeny appear as follows:

A. dom. + *B*. rec.	18.75%
A. rec. + *B*. dom.	18.75%
A. dom. + *B*. dom.	56.25%
A. rec. + *B*. rec.	6.25%

A result which again agrees with experiment. The resulting mixtures of characters in tri to polyhybrids may be likewise predicted, by extending the principles already laid down.

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REGENERATION OF PROPORTIONATE STRUCTURES IN STENTOR.

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THE important results of Gruber and of Balbiani on the power of regeneration of pieces of *Stentor coeruleus* opened the way for further experiments; and the works of Johnson and of Lillie on the same form have added some further results of interest. There remained, however, one problem that had not been touched upon by these investigators, an answer to which is needed to make more complete our knowledge of the regeneration of unicellular forms. I refer to the question of the proportionate development of the new organs in pieces of different sizes, and from different parts of the body; and also the no less important question of the change in size of old organs that may be present on the piece at the time of its removal. It is the purpose of the present communication to describe certain experiments that bear on these questions.

Although it is evident, in a general way, from the figures given by Gruber and by Balbiani that a small piece produces a smaller peristome than does a large piece, yet their figures do not show definitely that such is the case, and, in fact, it would be difficult to determine that such is the case from observations made on the swimming animals. The figures that have so far been published represent the new stentor as it appears while contracted or when swimming. To obtain sufficiently accurate data for the problems that I wished to examine, it was necessary to make the measurements and drawings from the stentor at rest when in a fully expanded condition. The object of my work was to find an answer to the following questions: 1. Do small pieces produce a new organism having the typical proportions of the normal; and does it make any difference in this respect as to the part of the stentor from which the piece is taken? 2. If a piece containing the old peristome is cut off, will it retain the old peristome, or absorb it and produce a new one of proportionate size? If the old peristome persists, will it decrease in size until it has assumed the typical proportions? 3. If a part only of the old peristome is left on a piece, will the missing parts be regenerated from it, or will a new peristome develop?

There appeared during January and February in one of the aquaria in the laboratory a large number of stentors, whose presence seemed to be connected with the appearance of vast numbers of vorticellas, on which they fed. The operation of cutting the stentor in two or more parts was carried out either by means of small scissors, or, in most cases, by a sharp scalpel. The latter operation is greatly facilitated by placing the animals in a dish of water, the bottom of which is covered by a layer of paraffin.

The following measurements give the length of the normal blue stentor and the greatest width of the peristome.

Length.	Width of Peristome.
2.8 mm.	.52 mm.
1.6	.46
1.4	.40
1.7	.50
1.7	.48
1.6	.44
1.9	.48

If a stentor is cut in two by a cross-cut, as indicated in Fig. 1, *A*, *a–a* (the anterior piece, *B*, being smaller than the posterior, *C*), the cut surfaces of each piece are closed almost instantly by the outer layer bending over the exposed part. Only a faint, clear line on the surface indicates where the cut has been made. The history of the anterior piece is as follows: In the course of an hour or two the piece becomes somewhat more pointed at the posterior end, and then fixes itself by a foot that appears at that end (Fig. 1, *B*). The posterior end now begins to draw out into a stalk, and after thirteen hours the piece has assumed the form shown in Fig. 1, *B*^{1}. The piece is still proportionately too broad for its length, for although the peristome has become reduced in size it is not as

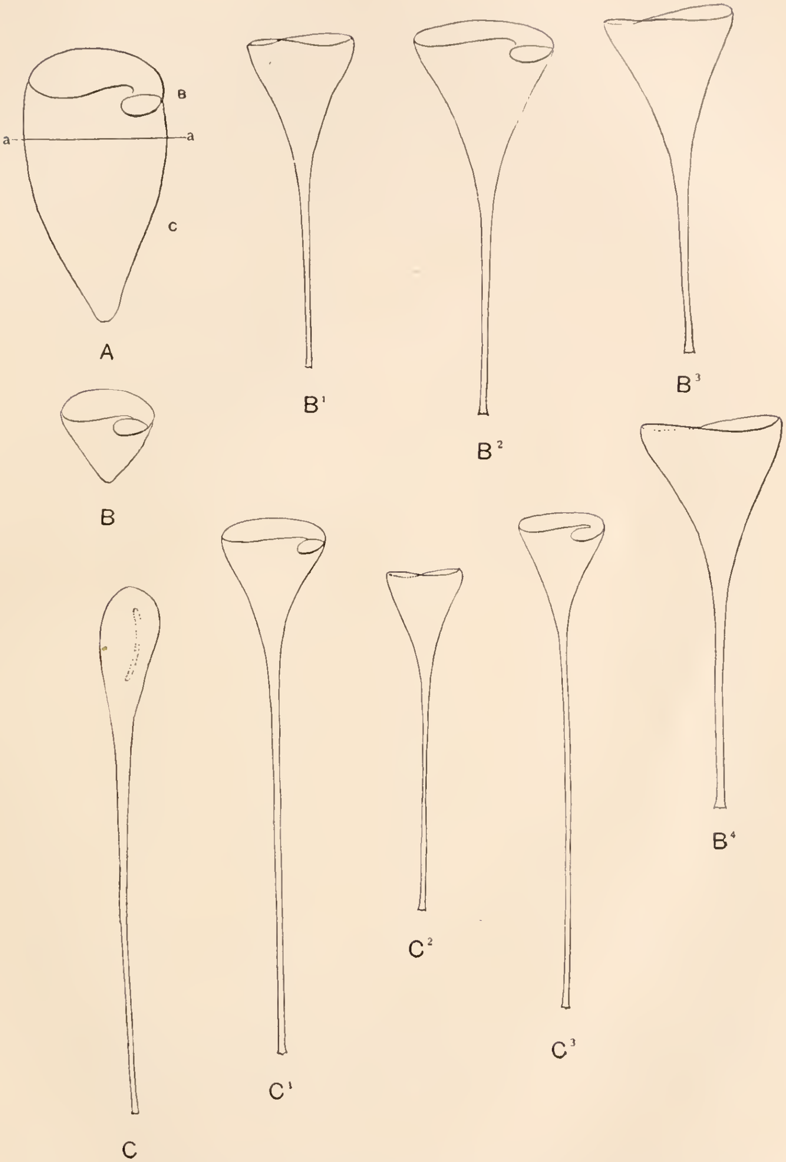

Fig. 1.—*A*, contracted stentor to show where the cut was made, *a-a*; *B*, anterior end five hours after the operation; B^1, an anterior piece thirteen hours after operation; B^2, B^3, anterior pieces twenty-four hours after operation; B^4, anterior piece forty-eight hours after the operation.

C, posterior or foot end of *A*; C^1, posterior end five hours after operation; C^2, C^3, posterior ends twenty-four hours after operation.

yet reduced sufficiently to give the piece the typical proportions. Two other head-pieces of this same series are shown in Fig. 1, *B*², *B*³, that were drawn twenty-four hours after the operation. It is even more evident in these (compare with Fig. 2, *A*, for normal) than in the last that the peristome is too broad for the length of the stentor. Even after another twenty-four hours one of the pieces had still retained the same form as shown in Fig. 1, *B*⁴.

The posterior piece, *C*, fixes itself at once by the old foot, and may soon elongate to its full length. In the course of two or three hours a clear band appears extending somewhat obliquely over the rounded end of the piece (Fig. 1, *C*). Cilia appear along the band. In a few more hours, the rate depending on the temperature, the ciliated band moves forward around the anterior end of the piece, and in doing so bends around on itself into the characteristic peristome. A new peristome-field, or disk marked by delicate parallel lines, appears on the inner side of the band even before it moves forward, and as the band bends around to make the terminal peristome, the new disk comes to lie in its central part. A depression, that appears at the basal end of the band, forms the pharyngeal funnel. The new peristome is smaller than that of the original animal, and, as the figures show (Fig. 1, *C*¹, *C*², *C*³), it is, in some cases, even smaller than the reduced peristome on the anterior piece. The foot-piece is also at first very long as compared with the size of the new peristome ; and this condition may remain for several days.

These results demonstrate that, for some time after the new organs have developed, the new stentors retain some of the peculiarities of the part of body from which they have come. When the pieces are contracted, or are swimming, these relations are scarcely evident and might easily escape detection.

The transformation of an anterior piece into a new stentor is much more strikingly seen when only a small part of the anterior end is cut off. One set of observations on the same individual is represented in Fig. 2. The stentor fully extended is represented in Fig. 2, *A*. The anterior end had been cut off, as shown in Fig. 2, *A*¹, while the animal was contracted.

After its removal the anterior end, *B*, contracted still more, so that its posterior cut-surface was quickly covered over by the bending in of the sides; the disk bulged forward. After a few hours the piece became somewhat pointed at its posterior end, and then fixed itself by a foot that appeared at the end.

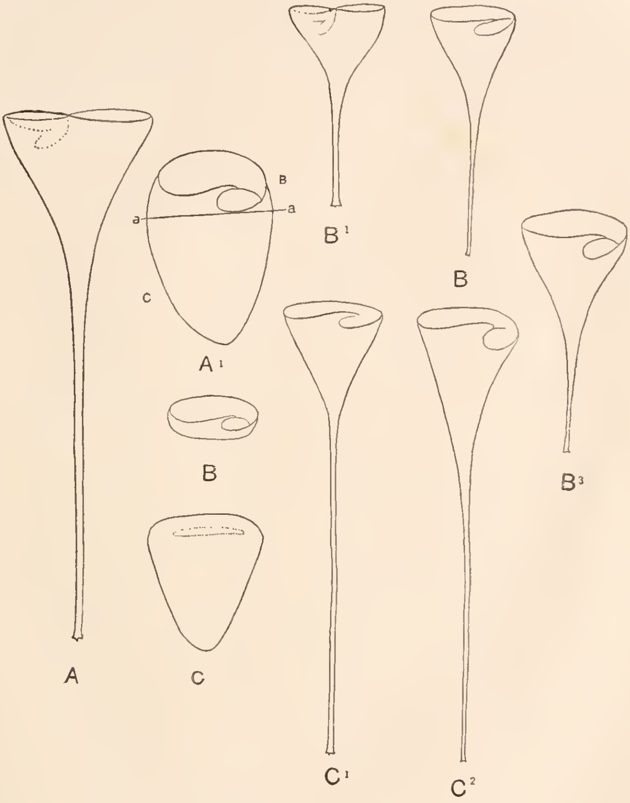

FIG. 2.—*A*, 1.4 × .4 mm. Stentor fully extended; A^1, same contracted, cut in two at *a–a*: *B*, *C*, immediately after operation; B^1, .56 × .26, anterior end after twenty-four hours; B^2, .7 × .25, same after another forty-eight hours; B^3, .64 × .28, same after another four days, *i.e.*, seven days after operation; *C*, 1.2 × .27, posterior end after twenty-four hours; C^2, 1.2 × .20, same after another forty-eight hours.

After twenty-four hours (it had been kept in the cold overnight, *i.e.*, for ten hours) it appeared as shown in Fig. 2, B^1. The old stentor measured 1.4 mm. by .4 mm. This new stentor measures .56 mm. by .26. The old peristome has, therefore, decreased nearly to half its original width. Two days later (Fig. 2, *B*^{2}) the stalk was somewhat longer, and after another four days (Fig. 2, *B*^{3}) the form had not materially changed. The development of the posterior piece of this same individual is shown in Fig. 2, *C*^{1} and *C*^{2}. The piece is about twice as long as the anterior piece, but its peristome is about the same size.

A similar operation was carried out on another individual; the results are shown in Fig. 3, *A*. A very small part of the anterior end was cut off (Fig. 3, *A*^{1}). It contained, however, the entire peristome and disk (Fig. 3, *B*). About thirty hours after the operation the anterior piece appeared as shown in

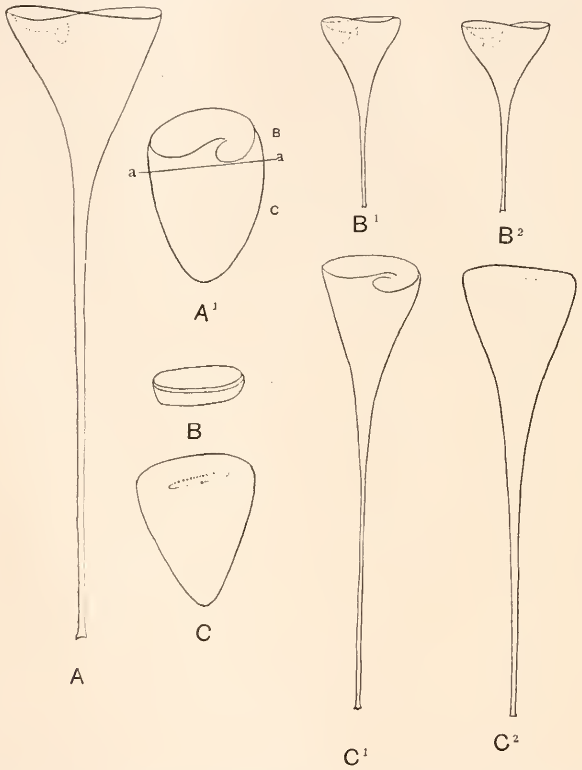

FIG. 3.—*A*, 1.7 × 4. Stentor fully extended; *A*^{1}, same contracted, cut in two at *a–a*; *B*, *C*, immediately after operation; *B*^{1}, .5 × .21, five hours after operation; *B*^{2}, .5 × .24, three days after operation; *C*^{1}, posterior piece, 1.2 × .27, five hours after operation; *C*^{2}, same, 1.6 × .32, three days after operation. In Figs. 2 and 3 one individual used in each. In Fig. 1 several individuals were used.

Fig. 3, B^1. After three days more the new stentor had about the same form. The peristome is, as compared to that of the original stentor, too wide for the length of the new individual, although it is not much more than half the width of the old peristome.

The development of the posterior piece is shown in Fig. 3, C^1 and C^2. In this piece, particularly in the earlier stage, C^1, the peristome is smaller than was the original peristome, and also relatively smaller as compared with the entire length of the animal.

In order to be certain that the anterior pieces did not produce new peristomes during the night, they were kept in a cold place when not under observation; for I had found that under these circumstances the formation of a new peristome is greatly delayed, even although it may have begun to develop before the piece is subjected to the cold. In this way I could retard the development of the peristome for twelve hours, so that I felt certain that a new peristome had not developed on these anterior pieces in my absence.

In other experiments pieces of different sizes were cut from the foot-end in order to see if the size of the new peristome that is formed is in proportion to the size of the piece. It was found that a smaller peristome develops on a smaller piece, and a larger one on a larger piece; and this same relation holds also for pieces of different sizes for other parts of the body. It has been shown that cross-pieces from the anterior or posterior ends retain some of their original peculiarities even after the formation of a new individual, and that for several days the stentors from anterior pieces are too broad for their length, and individuals from the posterior end are too long for their breadth. Some of these newly regenerated stentors from the anterior pieces were kept for a longer period and supplied with food. Their measurements for from one to seventeen days after the operation are given in the following table. The measurements of three normal individuals of this lot were 1.6 × .5; 1.4 × .4; 1.1 × .4. The experiment began February 3.

ANTERIOR PIECES OF HALF SIZE OR LESS.

Feb. 4.	.6 × .22
	.85 × .28
	.6 × .23
Feb. 6.	.6 × .23
	.6 × .3
	.68 × .2
	.7 × .25
Feb. 10.	.85 × .32
	.76 × .28
Feb. 13.	.7 × .34
	.8 × .35 × .25
	.9 × .28 × .2
	.64 × .28
	1.12 × .39
	1.2 × .28
Feb. 20.	.96 × .36
	1.00 × .39
	1.04 × .32
	1.00 × .40
	1.00 × .40
	1.00 × .39
	.9 × .36

It will be seen from this table that, after feeding, the stentors from anterior pieces grow larger. The increase takes place both in the peristome and in the length of the piece, so that the proportionate size of the disk to the rest of the piece remains about the same. The new stentors had begun to divide on February 13, and by February 20 there were about twice as many present as at first. They have, however, about the same proportionate size as at first. The question arises whether in the normal stentor the ratio of the breadth of the disk to the length of the pieces may not be less than in very large individuals. I measured some of the smaller individuals found in the aquaria with the larger ones and obtained the following results: 1.04 × .36; .72 × .26; .7 × .28; .72 × .3; .9 × .38 × .2; 1.1 × .28; .9 × .36 × .2; .98 × .28. There is seen to be some variation in the relative size of the length to the breadth; that is due in part to the individuals not always expanding to the same extent, and also in part to some of the measurements of the peristome not having been made in the widest part, but there are also actual differences, as some very careful measurements have shown. It will be seen that while in large stentors the greatest breadth of the peristome is about one-fourth, or nearly so, of the total length, in the small individuals the breadth is more nearly one-third of the length; therefore the peristome is proportionally somewhat larger for smaller pieces. Comparing these measurements with those of the sizes of individuals derived from pieces of the anterior end, we see that they have reached in several cases the characteristic form *for a small individual.* Since there is a good deal of variation in the proportion between the width of the peristome and the length of the animal both for small normal individuals and for those that have come from anterior pieces, it may be stated that pieces from the anterior end may produce new stentors whose proportions come within the range of variation of size of normal small stentors of about the same length. The measurements of posterior pieces, that are at first too long for the size of the peristome, show that they, too, assume more typical proportions. Thus one of the posterior ends of the last series measured, on February 6, 1.0 × .25. On February 10, two other individuals in the same list measured 1.4 × .38 ; 1.2 × .4. The peristomial region had, therefore, reached the full size.

A somewhat crude comparison may bring the results home. If a man were cut in two at the waist and the pieces behaved in the same way as those of stentor, two new individuals would develop. The anterior half would produce a small man with a head too large for his height, *i.e.*, his legs would be too short for a man with that sized head. Although the old head had grown smaller, it would be still too large for the rest of the new man. In fact, his proportions would be more like those of a baby whose head is relatively too large for his length as compared with that of a man, and his legs too short. It is just this result that we have found for anterior pieces of stentor. If the new man were supplied with food, all parts of the body would grow larger; but as he got larger his legs would grow faster than his head.

The posterior end of our imaginary man would have at first legs too long for his total length, and his new head would be relatively too small; but if he were fed his head, shoulders, and arms would grow faster than any other part and continue to grow until the proportionate size had been reached. If he were not fed, it is possible that his head and upper part might increase more slowly in size at the expense of the material in his legs, and the latter would get smaller until a balance was reached. The result would be that a boy rather than a baby was produced.

In other experiments pieces were removed that contained only a part of the peristome. In one series these pieces were cut off, as shown by the line *a–a* in Fig. 4, *A*; so that there was a smaller and a larger piece, the former, *B*, containing a part of the old peristome, but not any part of the pharyngeal funnel, and the latter, the larger piece, *C*, containing also a part of the peristome as well as the funnel. The cut surface of each piece is quickly closed by the bending in of the sides, and the cut ends of the peristome are generally brought together to make a closed ring. A foot develops on the basal end of the anterior piece, *B*, B^1, and a stalk is soon produced in that region. This piece may remain for twenty-four hours in this

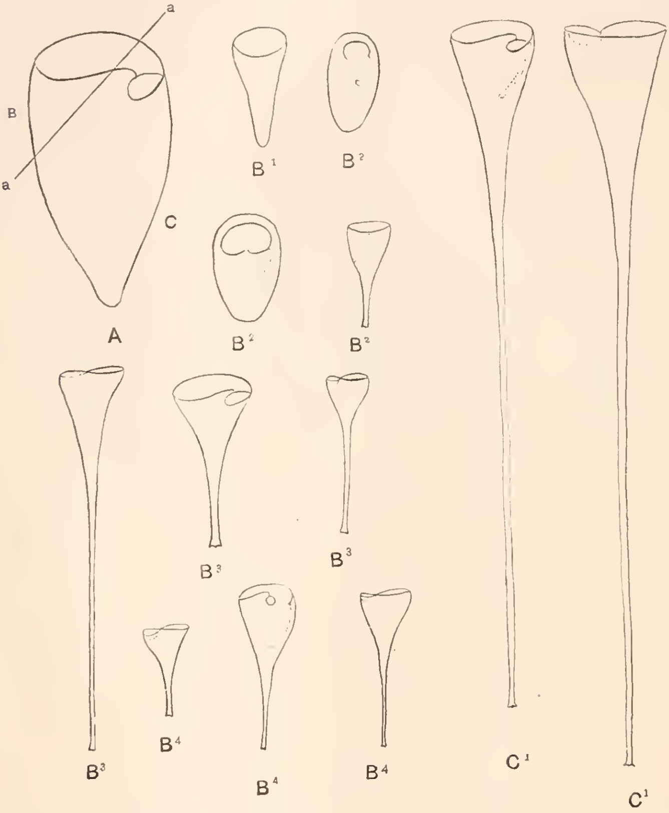

FIG. 4. — *A*, stentor partially contracted, cut in two at *a–a* into small piece, *B*, and larger, *C*: B^1, piece *B* after seven hours; B^2, B^2, B^2, piece like *B* after twenty-four hours; B^3, B^3, B^3, same after twenty-nine hours; B^4, B^4, B^4, same in part after forty-eight hours (after operation). C^1, C^1, piece *C* after seven hours.

condition. Sooner or later a new ciliated band appears on the old wall behind the part of the old peristome, as shown in Fig. 4, B^2. The band moves forward, fusing with the old ring at one point and, replacing the latter, produces a new peristome. Whether the piece of the old ring is entirely obliterated, or whether a part of it remains to contribute to the new peristome, I did not determine. The regeneration of a new peristome on these pieces may be delayed for several days (Fig. 4, B^4), and, in general, does not appear as soon as on pieces that do not contain any part of the old peristome. Five or six series of experiments of this sort, each series of a number of pieces, were made, and the smaller pieces followed with great care. Only those smaller pieces were isolated that contained no part of the old funnel. Nearly all the pieces behaved in the way just described, but in one or two a small funnel developed where the cut edges came together. This may have been due to a very small piece of the original funnel having been cut off, or to the piece having come from very near to the old funnel, or, as seems more probable, to the development of a new funnel from the old ciliated band. If the last interpretation is correct, it shows that in exceptional cases the peristome may complete itself. In the large majority of cases this does not occur and a new peristome and funnel develop at the side and move forward. In nearly all cases the cut ends of the old peristome come together, meeting in a slight notch. In one or two instances one band lay slightly below the other at the meeting point, producing a peristome exactly like the normal in shape, only the funnel was absent. If a small piece of the old funnel is left, it assumes the characteristic position of the funnel, and, in fact, becomes such to all appearance, although this peristome is generally replaced later by a new one.

Gruber studied the regeneration of pieces somewhat similar to these without a funnel, and states that the remaining part of the old peristome gives rise to a new one, but I have not found this to be the case. If the piece is kept under close observation, the development of a new peristome is found to take place in the way just described. The change is sometimes so rapid that a few hours may suffice to bring it about.

The history of the complementary piece (Fig. 4, *C*) is as follows: After the cut surface has been closed over and the edges of the peristome brought together, the piece may immediately fix itself by the old foot. The piece elongates to its full length, which is the same as that of the former animal (Fig. 4, C^1). In some of these pieces I have observed the development of a new peristome in the course of a few hours after the operation (Fig. 4, C^1). It seems that this takes place sooner when only a small part of the old peristome and funnel is left than when a larger part remains. In cases in which a large part of the old peristome remains a new peristome may not develop for several days; and in some cases I have not found it to appear at all, but I cannot state positively that it does not ultimately appear. Since even normal individuals may produce a new peristome, the appearance of a peristome on these new stentors after several days may be only the regular process of renewal of that organ. In two cases, in which a new peristome appeared after two days, the old one had begun to break down while the new band was developing. In all other cases the old part was still active and normal in appearance up to the time of its replacement by the new ciliated band. These results show that even in a large piece the new peristome is not regenerated from the old one. The presence of the old pharyngeal funnel in these pieces does not make any important difference in the end result, although it may be that pieces of this sort regenerate less quickly than when the piece does not contain the funnel portion of the old peristome.

Another experiment that supplements the preceding one in several respects consists in cutting the stentor in two, as indicated by the line *a–a* in Fig. 5, *A*. In this case the smaller piece, *B*, contains the funnel part of the peristome, while the larger piece, *C*, contains the remaining part of the peristome.

The smaller piece, *B*, closes in, develops a foot, becomes attached and produces a stalk. The edges of the peristomial ring unite more or less, as shown in Fig. 5, B^1. In one case a new peristomial band appeared six hours after the operation, moved forward, and produced a new peristome (Fig. 5, B^1). In other cases, in which the piece was small in comparison to the size of the remaining part of the peristome, a new peristome did not appear in one case until after thirty hours (Fig. 5, B^4); in other cases a new peristome had not appeared at this time (Fig. 5, B^3).

The complementary piece (*C*, Fig. 5) closed in, fixed itself, and extended to its full length. In pieces in which the remaining part of the peristome, that had united to make a ring, was quite small a new ciliated band appeared in four hours; in others, in six hours (Fig. 5, C^1 C^1); and in pieces with a larger peristomial region, after twenty-four and even after fifty-one hours (Fig. 5, C^3). It is interesting to note that in these pieces the region from which in the normal individual the peristomial band is formed has been more or less completely removed, yet a new peristomial band may very quickly

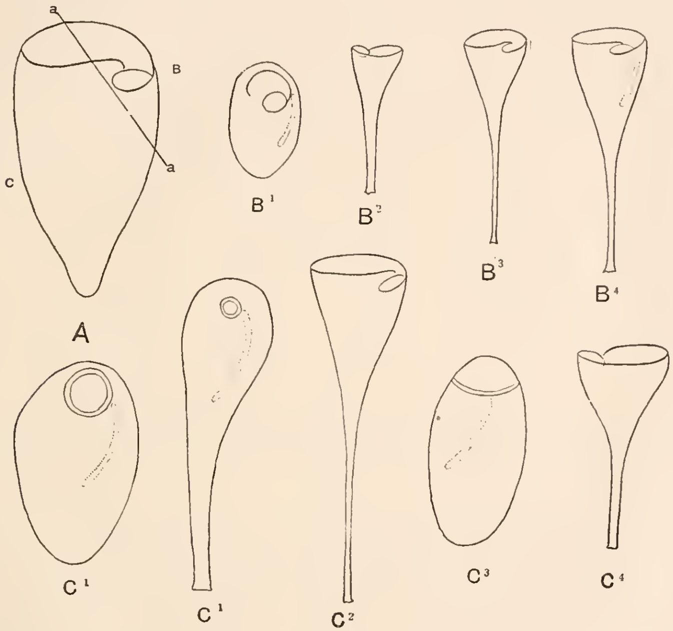

FIG. 5.—*A*, stentor partially contracted, cut in two at *a–a* into a small piece, *B*, and a large piece, *C*; B^1, piece like *B* after seven hours; B^2, piece like *B* after twenty-seven hours; B^3, B^4, piece like *B* after twenty-nine hours; C^1, C^1, piece like *C* after seven hours; C^2, piece like *C* after twenty-nine hours; C^3, C^4, piece like *C* after fifty-one hours.

appear. I did not attempt to determine the position of the new band in its relation to the region of closure of the piece.

In several cases in the last two experiments, and in some other experiments like those shown in Fig. 6, *A*, B^1, C^1, small pieces were sometimes cut off that contained a part of the old peristome, but which did not fix themselves, or assume the characteristic form. As these pieces were generally small, although not below the minimal size, there can be little doubt that most of them did not contain any part of the nucleus, and in several cases I proved this to be the case by staining the pieces in picro-carmine. The result shows that in the absence of the nucleus a piece containing a part of the old peristome cannot complete the peristome from the remaining part. This is the less to be expected since it has been shown that even in nucleated pieces the new peristome is produced not by the old one, but by the development of a new peristomial band. The result is interesting in connection with a result obtained by Gruber, *viz.*, that if a non-nucleated piece containing a part of the newly forming ciliated band is obtained it produces from the band a new peristome. My results show that a piece of the old band cannot act in this way. That the presence of the nucleus is connected with the formation of a new peristomial band seems highly probable, but I can easily imagine that could a non-nucleated piece be supplied with certain unformed elements it might be capable of producing a new peristome. The results do not seem to me to show more than that the nucleus supplies certain products of metabolism that must be present before the protoplasm can successfully carry out its

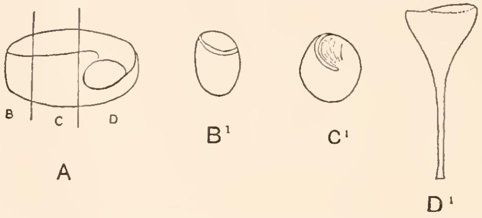

Fig. 6. — *A*, anterior end of stentor cut off and then divided into three pieces. Two of these, B^1, C^1, were apparently without nuclei, and did not produce a new peristome or assume the typical form.

innate tendency to complete the typical form. We are not justified, I believe, in drawing the conclusion, as Gruber has done, that preformed elements of the peristome exist in the nucleus and must be set free in order to initiate the development of a new peristome.

Lillie has found that the smallest piece of *Stentor polymorphus* that becomes a perfect form is equal to a sphere of about 80 μ in diameter. The average size of the stentors was equal to 230 μ. This makes the volume of the smallest stentor about $\frac{1}{27}$ of the normal. For *Stentor coeruleus* the smallest stentors measured 90 μ ($=\frac{1}{11}$ mm.), the average normal stentor 280 μ ($=\frac{7}{25}$ mm.). Therefore the former is about $\frac{1}{27}$ of the latter.

Although I have not worked specially on this problem, yet I have obtained some small stentors that were proportionately smaller than those obtained by Lillie. Thus one individual measured when extended .25 × — .08 mm., and when contracted into an oval or nearly into a sphere .08 × .08 ($=\frac{1}{12}$ mm.). The larger normal stentors measured about .4 × .32 × .32 when contracted. Although it is only possible to give a general estimate of the relative size of these two individuals, the smaller cannot be over $\frac{1}{64}$ of the former. It would be a mistake to infer from this, as well as from Lillie's calculations, that the latter came from a piece $\frac{1}{64}$ or even $\frac{1}{27}$ of the original stentor. The protoplasm of stentor is so vacuolated that a piece losing the fluid in the protoplasm might become much smaller than when first removed.

Lillie states that he believes that it would be possible to obtain a smaller individual of *S. coeruleus* than $\frac{1}{11}$ mm. The one that I obtained was in fact somewhat smaller, *viz.*, $\frac{1}{12}$ mm. The difference in our results depends, therefore, rather on the size of the normal average stentor with which the comparison is made than on the smallest individual obtained. Lillie says that he does not think there can be much difference in the absolute size of the smallest stentors, whether one uses the largest or the smallest normal specimens. It seems to me that this may or may not be true, according to what factors may enter into the result.

The conclusion that a piece $\frac{1}{27}$ or even $\frac{1}{64}$ of the entire animal can produce a new individual can give only a most general idea of the relative size of the smallest piece, since more depends on the size of the normal individual than on that of the smallest pieces, and there is for stentor a very wide range of size that may be called normal. A large normal individual may contain eight times the volume of a small normal individual. More significant, therefore, is the absolute size of the smallest piece capable of regeneration, and in this respect my results are practically in accord with those of Lillie.

Several experiments were made in which pieces were cut in two longitudinally. In a longer or shorter time most of the halves produced a new peristomial band that became a new peristome. As this experiment did not give promise of much that is new, it was tried in only a few cases.

A few casual observations made during the course of the work may be briefly mentioned. The stentors were observed dividing on several occasions, but Johnson's excellent figures and account of these stages leave nothing new for me to add. I have often noticed that after division the two products are found attached side by side, and if they are not disturbed a little colony may arise in the same spot. Several times I have observed that two individuals that have been formed by division of one of the regenerated posterior pieces were unequal in size, although I do not know whether the smaller individual was the distal or the proximal one.

As Gruber has pointed out, the first steps in the process of division and of regeneration are the same, and this holds also for the physiological replacement of the old peristome. In all cases a peristomial ciliated band appears *on the side* and moves forward around the anterior end to become the new peristome. We have here another illustration that shows that during the process of regeneration the factors that appear in the normal growth may take part in the regeneration, and this relation appears to hold for unicellular as well as for multicellular forms.

In many cases, especially where a somewhat oblique cut has been made, the superficial blue stripes come together over the cut surface in a most irregular way, yet this does not appear to interfere with the subsequent regeneration ; and after a time the stripes appear to be more regularly arranged. That a certain amount of absorption takes place, and possibly also development of new stripes, seems probable, but I have not studied these changes in any detail. It would be interesting to find out if in cross-pieces of the body the number of the stripes remains the same and their size becomes smaller, or whether the number of the stripes is proportionately reduced.

On several occasions I have tried to graft together pieces of different stentors, but the exposed surfaces close so quickly that I have not been able to get the pieces to unite. It does not seem altogether improbable that the result could be brought about by cutting two stentors at the same time, one about the other. A lucky cut might bring two exposed inner surfaces together, and they might stick to each other, but so far I have not been able to carry out successfully this experiment.

In a few cases the stentor was split partially in two pieces, but generally the halves soon fuse together. It is of some interest to find that, although the peristome was cut in two and had reunited, a new peristome was not produced, showing that the operation alone does not initiate the changes that lead to the development of a new peristome.

The development of a new peristome in a piece that contains a part of the old one appears to be due to the lack of proportion between the old part (even when it contains all the essential parts of the peristome) and the rest of the piece. This result is unique, since in all other forms in which a part of an old organ remains the new organ regenerates from that part. In stentor this does not occur, but a new organ is produced. It is important to observe, however, that this is the characteristic way in which stentor produces a peristome, so that the organisms make use of a process that already exists. The reduction in size of the old peristome in pieces from the anterior end is the result that has most interested me. It seems to be due to the withdrawal of material from the anterior region to form the body and stalk of the new stentor.

The change in shape of the piece, *i.e.*, the production of the typical form, is primarily the result of a shifting of the material that carries with it a loss of material in the old part. Other so-called formative factors may have some share in the reduction in size of the old peristome to one proportionate to the rest of the piece, but the simple loss of material will, I think, account for the greater part of the change.

What primarily brings about this change in the material so that the typical form is produced is a question to which at present there is no answer.

BRYN MAWR, March 12, 1901.

ABSTRACTS OF PAPERS

PRESENTED AT THE MEETINGS OF THE

AMERICAN MORPHOLOGICAL SOCIETY

At Baltimore, December 27 and 28, 1900.

I. FISSION AND REGULATION IN STENOSTOMUM LEUCOPS.

C. M. CHILD.

The single individual of *Stenostomum* differs considerably in size, according to conditions. Well-nourished specimens may reach a length of nearly one and one-half millimeters, while specimens measuring only one-half millimeter are often found when food is scarce. The length is about eight or ten times the transverse diameter.

The animals usually occur in chains, the number of zoöids varying from two to nine. Ordinarily chains do not consist of more than five zoöids, the uneven number being due to the fact that the anterior zoöid precedes the others in division. The short chains of two or three zoöids occur when food is scarce. In well-nourished specimens the fissions succeed each other more rapidly, and longer chains are the result. Each particular septum occurs, with little variation, in a definite, characteristic position, this apparently being determined largely by the relative degree of development of the two ends of the zoöid which it divides.

Entodermal tissue is necessary for regeneration. Portions containing all the other tissues of the body except entoderm do not regenerate, but if a small portion of the entodermal digestive sac be present regeneration is complete, provided the piece is above a certain size.

If a chain be artificially separated into its zoöids before they have attained their full development, each zoöid undergoes a form-regulation, assuming within a few hours what may be called the normal proportions, *i.e.*, the length becomes eight or ten times the transverse diameter. This regulation does not occur while the individual is a zoöid in a chain, because the whole chain is not simply a series of individuals, but in some degree a single individual, and therefore possesses certain proportions differing from those which each zoöid would possess if single.

When the chain is cut at various points between the zones of fission, the results differ according to the degree of development of the particular zone of fission concerned and the parts adjoining it. If a piece containing a very young septum be cut out from a chain, the septum disappears and a single perfect individual is formed from the parts, which originally belonged to two different zoöids. A head is regenerated at the anterior cut surface, a tail at the posterior end. If the included septum be more fully developed, it remains, and the part anterior to it is completely absorbed by the part posterior to it, the head of the new individual resulting being the head which was forming just posterior to the septum. The relative size of the parts anterior and posterior to the septum does not affect the result, unless the posterior piece be very small. It is always the anterior part which is absorbed, never the posterior. If the septum be still more advanced in development, the portion anterior to it is only partly absorbed. It regenerates a new head and becomes a perfect zoöid, though at first it decreases in size, owing to the partial absorption.

In general each zoöid tends to absorb material from the zoöid anterior to it. Each zoöid, however, offers a certain resistance to this absorption, the resistance increasing as it approaches the condition of independent individuality. When the individuality of a zoöid is destroyed or reduced to a lower degree, *e.g.*, by cutting it in half, the posterior half may be completely absorbed by the zoöids posterior to it.

The sexual individual arises as a zoöid in a chain, but when the sexual organs appear, asexual reproduction ceases. The single sexual individual may, however, attain a length equal to that of the longest chains. The power of regeneration is much less in the sexual than in the asexual condition. Apparently in the former the energy is chiefly directed toward the elaboration of the sexual products.

II. THE OCCURRENCE OF GUNDA SEGMENTATA IN AMERICA.

WINTERTON C. CURTIS.

A SPECIES of *Gunda*, which in its external features seemed identical with the *G. segmentata* of Lang, was found in large numbers at Sandwich on Cape Cod. The internal arrangement is not, however, as regular as Lang describes for *G. segmentata*. From a comparison with Verrill's figure of *Procerodes ulvae* collected in the same region (*Trans. Conn. Acad.*, Vol. VIII, January, 1893), it is probable that the two forms are identical and that Verrill has figured the head incorrectly.

III. SOME DISPUTED POINTS IN THE ANATOMY OF THE LIMPETS.

M. A. WILLCOX.

THE following points were made:

1. The space previously described by Willcox as the nephridium is lined throughout by more or less columnar cells provided with long, delicate cilia and loaded in the fresh condition with dark green granules. The histology lends no countenance to Haller's contention that the posterior part of this sac represents coelom, the anterior nephridium.

2. The space which in most species of *Acmaea* underlies the viscera on the left side in *A. testudinalis* stretches almost across the body, and lacks entirely the ciliated cells characteristic of the nephridium. This negatives the opinion that the space in question is a paired structure whose fellow of the right side is represented by the posterior part of the nephridium.

3. A subradular organ, whose presence in the Docoglossa has been denied, exists in both *A. testudinalis* and *A. fragilis.* It is situated on the underside of the odontophore, just behind the tip of the radula, and is a triangular, somewhat cushion-shaped organ, divided by a V-shaped groove into an anterior and a posterior part. The posterior part is marked by transverse grooves and is covered by tall, columnar epithelial cells, some of which seem to be ciliated, while others are somewhat fusiform and have much the appearance of sense cells. The innervation has not been traced, but no ganglia are to be found in the organ. The subepidermal portion consists of connective tissue with scattered and inter-crossing muscular fibers.

IV. THE HABITS AND LIFE HISTORY OF ARGULUS WITH REFERENCE TO ITS ECONOMIC IMPORTANCE.

CHARLES B. WILSON.

In the town of Warren, Mass., is a small pond which was stocked with carp and bass several years ago. The fish seemed to thrive well until the fall of 1899, when they began to die off in considerable numbers, with no apparent signs of disease or injury. No clue to the cause of the devastation could be obtained till the spring of 1900, when several suckers were speared in the outlet of the pond whose gill chambers were full of the parasitic copepod *Argulus*, probably *A. catostomi.* The gentleman who owned the pond stated that these copepods were common on most of the fish caught there, and his statement was afterward verified. On being put in an aquarium with dace, roach, and bream, they attacked these fish viciously and the next morning they were found dead. *Argulus* deposits its eggs instead of carrying them around like other crustaceans, arranging them in rows on sticks, stones, etc., with their long diameters parallel.

When laid they are covered with a jelly envelope consisting of beads of jelly arranged in rows parallel with the long diameter of the egg. These harden into a brittle shell. The eggs are fertilized outside the body of the female and there is no copulation. The egg hatches into a typical nauplius, which after one or two moults changes into a metanauplius having highly developed clasping organs in the shape of barbed claws terminating the second maxillae. On putting two small dace into the aquarium with about two hundred of these larvae, the latter made no attempt to use their claspers, but the fish, on recovering from their fright, ate up every one of the larvae. On inquiry it was found that the pond in question had been seined for three years, and that the dace and roach had been sold for pickerel bait. *First conclusion*, the subject of parasitism is not so one-sided as would appear at first sight. *Second conclusion*, the protection of small fish like dace and roach in our fish ponds may be one of the best preventives against such parasites as these.

V. THE ANATOMY AND DEVELOPMENT OF THE POSTERIOR VENA CAVA IN DIDELPHYS VIRGINIANA (KERR, LINN.).

C. F. W. McCLURE.

An examination, thus far, of forty-eight opossums has brought to light many interesting variations concerning the mode of formation of their posterior vena cava.

These variations are so pronounced and so closely accord with certain embryonic conditions described by Hochstetter for *Echidna aculeata*, it seems to the writer as not improbable that the development of the posterior vena cava may take place in *Didelphys* and *Echidna* in substantially the same manner.

In all marsupials hitherto examined (*Petaurus taguanoides* excepted) the posterior vena cava has been found to lie ventral to the aorta between the renal and common iliac veins, and to be formed through a union of the common iliac veins, which takes place *ventral* to the arteries.

In *Didelphys virginiana* the posterior vena cava is not formed in this manner.

In fact, the mode of formation of the posterior vena cava was found to be so variable in *Didelphys virginiana* that it is quite impossible to assign any one mode of origin for this vessel which may be regarded as typical of the species.

For descriptive purposes the various modes of origin of the posterior vena cava in *Didelphys* have been classified by the writer under three main types as follows:

Type I includes those cases in which the internal iliac veins unite with the external iliac veins to form the posterior vena cava, *ventral* to the common iliac arteries or *ventral* to the aorta.

Type II includes those cases in which the internal iliac veins unite with the external iliac veins to form the posterior vena cava, *dorsal* to the common iliac arteries or *dorsal* to the aorta.

Type III includes those cases in which the internal iliac veins unite with the external iliac veins to form the posterior vena cava, both *dorsal* and *ventral* to the common iliac arteries or both *dorsal* and *ventral* to the aorta.

So many variations of this type were met with that a further subdivision of Type III was found necessary, as follows:

Type III, A, includes those cases in which the principal union between the internal and external iliac veins lies *ventral* to the arteries in question.

Type III, B, includes those cases in which the principal union between the internal and external iliac veins lies *dorsal* to the arteries in question.

Type III, C, includes those cases in which the abovementioned *dorsal* and *ventral* unions are *sub-equally* developed.

The following table shows how the above-mentioned types were distributed among the forty-eight individual opossums examined (twenty-four males and twenty-four females).

Type.	♂	♀	Total.	
Type I	5	9	14	14
Type II	6	7	13	13
Type III *A*	3	2	5	21
B	8	5	13	
C	2	1	3	
Total	24	24		48

A comparison with the development stages in *Echidna aculeata* shows, I think beyond the question of a doubt, that the variations in the method of formation of the posterior vena cava in *Didelphys*, so far as its posterior tributaries are concerned, are modifications of a ground plan arrangement similar to that described by Hochstetter for his *Echidna* embryo No. 45.

The writer's investigations upon the development of the posterior vena cava are as yet incomplete. So far as they have gone, however (an examination of five 15-millimeter embryos), they decidedly favor the above conclusions.

VI. THE CROSSING OF THE OPTIC NERVES IN TELEOSTS.

G. H. PARKER.

In ten species of symmetrical teleosts, in each of which one hundred specimens were examined, the right optic nerve was dorsal at the crossing about as frequently as the left. The two types of crossing (right nerve dorsal and left nerve dorsal) were not correlated with sex and were about equally frequent in specimens taken from one school of fish. In one hundred specimens of the winter flounder (*Pseudopleuronectes americanus*), whose eyes are on the right side, all had the left nerves dorsal. In seventeen specimens of the summer flounder (*Paralichthys dentatus*), whose eyes are on the left side, all had the right nerves dorsal. In one hundred specimens of the stellate flounder (*Platichthys stellatus*) all had the left nerves dorsal, notwithstanding the fact that fifty of these fish had their eyes on the right side and fifty on the left. Although each species of symmetrical teleosts examined showed about equal numbers of the two possible types of optic nerve crossing, the flounders showed only one type for each species.

VII. A NEW TYPE OF BUDDING IN ANNELIDS.

H. P. JOHNSON.

Two gigantic undescribed species of Pacific coast *Syllidae* produce reproductive zoöids by collateral budding from a definite proliferating region near the posterior extremity.

The single specimen of the larger species (*Trypanosyllis ingens*, sp. nov.) at my command was too poorly preserved for thorough study, but what I have learned about its budding agrees essentially with the fuller knowledge acquired from the other species. The buds numbered about thirty, all turgid with nearly ripe ova. No very young buds were detected, as in the succeeding species.

Of the other species (*Trypanosyllis gemmipara*, sp. nov.) I have several specimens, but only one with buds. They develop from a proliferating region twenty somites anterior to the pygidium. The advanced buds are broadly elliptical, and much flattened dorso-ventrally. Each is attached at its head-end by a short pedicle. The somites number 20–28, with parapodia which are miniatures of those of the parent. The prostomium has large eyes, a pair of antennae, and brain. There are a pair of ventral nerve cords, a muscular system, septa, and large paired masses of sperm cells in every coelomic chamber from prostomium to pygidium. Purely vegetative organs (*e.g.*, mouth, alimentary canal, anus, and nephridia) are absent, although a rudiment of the alimentary canal may exist as a median strand of tissue extending the length of the bud.

The youngest buds form a cluster of about twenty-five attached to the right side of the zone of proliferation, on its ventral aspect. The earliest-formed organs are the anal cirri, at first two distal protuberances which elongate and become moniliform before the bud segments. Apparently the bud contains only ectoderm and mesoderm, which are continuous with the same germ layers of the proliferating region.

In neither species are there any reproductive cells in the body of the parent anterior to the proliferating region, but sperm cells are present in *T. gemmipara* in the twenty parental somites back of this point.

VIII. AMPHIBIAN STUDIES.

J. S. KINGSLEY.

THE following are the chief points made in the paper:

1. The *Salamandrina* form the central Urodele stem, and the Perennibranchs and Derotremes have been derived from this stem by degeneration and the retention of larval characters.

2. The Urodeles cannot have been the ancestors of the *Anura*; the anuran tadpole resembles the Urodele only in superficial characters; the *Anura* have descended directly from the *Stegocephala*.

3. *Amphiuma* has no tentacular apparatus at any stage; what was described as such by Davison was a trematode parasitic in the suborbital blood vessel.

4. The Caecilians differ from all Urodeles in the fact that the palatine nerve receives a branch from the ophthalmicus profundus instead of from the maxillaris superior nerve.

5. The Caecilians have not descended from the Urodeles, nor is *Amphiuma* a neotaenic Caecilian. The Caecilians are degenerate in loss of limbs and tail; in all other respects they are the most primitive of living *Amphibia*.

6. No Stegocephalan as yet known can have served as ancestor of Urodeles, *Anura*, or Caecilians. The parent form must have possessed characters intermediate between the known *Stegocephali* and the Crossopterygian ganoids.

7. The ancestry of the *Amphibia* must be sought in the *Crossopterygii* and not in the *Dipnoi*.

8. The balancer of the Urodele larva is a modified external gill belonging to the hyoid arch.

IX. PHAGOCYTOSIS IN A MAMMALIAN OVARY.

MAYNARD M. METCALF.

IT has long been known that in the ovaries of certain Mammals and Fishes syncytia of young ova are found, and that of the several nuclei in such a syncytium one or but few persist as nuclei of definitive ova. Apparently, in these cases, the cells which disappear are used as food for the persistent ova.

In the ovary of the common Cat somewhat similar conditions have been observed by the author. Many of the young ova, in the stage when they are surrounded by a follicle consisting of but two layers of cells, are seen to have ingested many of the follicle cells, and the nuclei of these ingested cells can clearly be seen, some quite perfect (newly ingested), others apparently in different stages of digestion. The nuclei of these ingested cells, when almost completely digested, appear as groups of granules, these granules being apparently the remnants of the nodal thickenings of the chromatin network of the ingested cells. Such an ovum with its ingested nuclei very closely resembles the young blastomeres of a *Salpa* embryo, which have the same habit of devouring follicle cells.

Many young ova with ingested follicle cells were found in one Rat ovary. In another ovary of a White Rat no ingestion of follicle cells was found, nor was ingestion found in the ovary of a Gray Squirrel examined. Pressure of other duties has prevented the author from determining if such ingestion of follicle cells be normal in the ova of Rats and, if so, what relation, if any, it may have to the age of the individual or to its condition as regards reproduction. The observations are reported in the hope that other observers may have them in mind. If the phenomena are at all general among *Mammalia*, they should be seen in many laboratories in the usual histological demonstrations.

Similar phenomena are, of course, well known in several groups of *Invertebrata*.

X. THE MAMMALIAN LOWER JAW.

W. H. RUDDICK AND J. S. KINGSLEY.

In no adult mammal, recent or fossil, is the lower jaw known to consist of more than a single bone, and no author, save W. K. Parker, whose observations appear to have been overlooked, has shown the existence of more elements in its development. We are able to confirm Parker in his account and to identify in the mammals the following bones of the non-mammalian groups: (1) articulare, (2) angulare, (3) splenial, (4) dentary. Of these, articulare and angulare unite to form the malleus, while the definitive lower jaw is composed of dentary and splenial. Two cartilages participate in the formation of the lower jaw, — the Meckelian and a second larger cartilage lying external to this, which, like Parker, we homologize with one of the lower labials of the *Ichthyopsida*. In its ossification this cartilage is strikingly similar to amphibian cartilages, and the resulting bone — a part of the dentary — gives rise to the posterior part of the lower jaw, including the coronoid and articular processes. In the existence of this lower labial is to be found the explanation of the shifting of the articulation of the lower jaw. It is noteworthy that a lower labial occurs in about the same position in the ganoid *Polypterus*.

XI. AN APPARATUS IN THE CENTRAL NERVOUS SYSTEM OF VERTEBRATES FOR THE TRANSMISSION OF MOTOR REFLEXES ARISING FROM OPTICAL STIMULI.

PORTER EDWARD SARGENT.

In *Amia*, at about the time of hatching, there arises in the anterior portion of the roof of the optic ventricle a group of cells, eighty to one hundred in number, formed by the differentiation of indifferent neuroblasts. During the first and second days of larval life the axons develop from these cells as exceedingly fine processes, growing directly toward and into the optic ventricle. Early in the third day the adjacent axons come together in groups and coalesce at their tips, in their further growth through the cerebro-spinal fluid appearing as a single fibril. Later these fibrils coalesce with others similarly formed, and in their growth posteriorly through the ventricles and canalis centralis form what has been known as Reissner's fibre, which is then a fibre tract made up of many axons closely united and surrounded by a single medullary sheath. Through the posterior portion of its course there come off from it fine fibrils which pass through the canal obliquely backwards and enter the tissue of the cord.

In the first day after hatching there may be found in the extreme posterior end of the canalis centralis a number of small cells, three to four micra in diameter, lying in the lumen of the canal and ventriculus terminalis. Some eight to ten of these cells persist and continue to develop. Increasing rapidly in size, they become spindle-shaped and send their axons cephalad through the canal. The axons are at first separate, but later coalesce as they grow forward, and, eventually meeting the system of axons from the cells of the tectum growing posteriorly, the two interweave in a way not yet clearly made out.

The development of this apparatus in *Amia* is typical of its development in all vertebrates, though in some groups there are considerable variations. In the Skate the cells are of conspicuous size, and three to four hundred in number. They are multipolar, sending processes to the ectal portion of the tectum, where they come in direct contact with the endings of the optic nerve fibres and give rise to two fibre tracts, one of which passes posteriorly to the cerebellum, the other anteriorly and out into the optic ventricle to form the Reissner's fibre. In reptiles and birds the apparatus appears at a late stage and is not fully established until just before hatching.

In *Petromyzon* this apparatus is not fully established until the second month of larval life. The cells, about twelve in number, form a well-marked nucleus. Reissner's fibre passes through the optic ventricle, reënters the brain tissue, and again emerges into the fourth ventricle. Thus *Petromyzon* furnishes the connecting link between the condition in the Gnathostomes and *Amphioxus*. In the latter the largest and most anterior of the colossal dorsal cells lies across the central canal, is in direct connection with the pigment spot, and sends its axōn caudad through the cord in the median plane just ventral to the canal. This axon probably represents Reissner's fibre.

There are many lines of evidence which lead me to assign the function I have to this apparatus.

1. The cells are in direct connection with the endings of the optic nerve, and with the cerebellum. The axons pass by the shortest path posteriorly through the central canal, and probably out through the ventral roots to the musculature.

2. When the fibre is cut in Sharks or Dogfish they evidence an inability to respond quickly to optical stimuli.

3. In the vertebrates of the cave fauna the apparatus degenerates as the eye degenerates.

4. In no animal does the apparatus reach complete development until just before the animal attains free life.

5. In those animals which are sluggish at hatching (*Petromyzon*, *Amia*), the apparatus is not fully developed until a considerably later period.

6. In those mammals which are born blind (Mouse, Kitten), the apparatus is not fully established until about the time the eyes become functional.

7. In any one group, as the Teleosts, the apparatus has its highest development in those species which are most active.

8. The corpora quadrigemina of higher vertebrates are concerned only with reflex functions; therefore this apparatus must have a reflex function.

Such a short circuit avoiding the loss of time in passing through a chain of neurones must be of great importance in saving time, amounting perhaps to a considerable fraction of a second. An animal suddenly presented with some optical evidence of danger from which it recoils in fear, does so reflexly, calling into use this apparatus. When we consider that in the struggle for existence the saving of a fraction of a second is often a matter of life or death, it becomes evident that this apparatus has played an important part in the survival of the fittest, and in the whole evolutionary process throughout the vertebrate series.

XII. THE SIGNIFICANCE OF THE SYNAPSIS STAGE OF THE GERM CELLS.

THOS. H. MONTGOMERY, Jr.

In the germinal cycle of the *Metazoa* may be distinguished in succession the following main stages: the conjugation of the maternal and paternal cells (fertilization), a number of generations of ovogonia (or spermatogonia), then the growth period, and finally the stage of the two maturation divisions. The reduction in the number of the chromosomes, *i.e.*, the formation of bivalent chromosomes, is not effected by either of the maturation mitoses, but during that portion of the growth period known as the synapsis stage. The bivalent chromosomes are formed by a union, end to end, of every two univalent chromosomes, as I have shown in a paper on the spermatogenesis of *Peripatus*, just published, and in another on the spermatogenesis of the *Hemiptera*, now in press.

Heretofore no one has shown exactly how the bivalent chromosomes are produced, and no one has given any adequate explanation for the reason of their formation. My comparative studies on the spermatogenesis of a considerable number of species of *Hemiptera*, which have brought to light certain facts of importance for determining these questions, render it probable that in the process of formation of the bivalent chromosomes we have a conjugation of paternal with maternal chromosomes. This would then be the final stage in the fertilization of the germ cells; it would be a conjugation of the chromosomes of different parentage producing a rejuvenation of them as metabolic centers of the cell; and this rejuvenation finds its expression in the great changes of the growth period. Then, probably, the reduction division takes place, in order to separate again the conjugating chromosomes, as two conjugating *Infusoria* unite and then separate after the accomplishment of the rejuvenescence.

In the space of a short abstract it is not possible to give the evidence for these conclusions.

XIII. A STUDY OF THE PHENOMENA OF FERTILIZATION AND CLEAVAGE IN ETHERIZED EGGS.

EDMUND B. WILSON.

A.

If fertilized eggs of *Toxopneustes*, after the formation of the cleavage-figure, be placed in a 2.5% solution of ether in sea water, the astral rays quickly fade out, as was long since observed by O. and R. Hertwig in sea-urchin eggs treated by solutions of chloral hydrate or sulphate of quinine. The clear hyaloplasm masses forming the astral centers are thus left as well-defined, slightly irregular, non-radiate areas, connected by the spindle-area. If the eggs are replaced in sea water, the rays are rapidly redeveloped and cleavage may proceed nearly or quite normally. Even if left in the ether solution, however, the nuclear division may be completed, the daughter-nuclei being re-formed and growing to their normal size, *but no cytoplasmic division occurs*, — a result similar to the earlier ones of Demoor on the division of plant cells *in vacuo*, or in an atmosphere of CO_2, and to those of Loeb and Norman on *Arbacia* eggs in sea water concentrated by the addition of a small percentage of chloride of sodium or magnesium. If the strength of the ether solution be now somewhat reduced, by evaporation or by the addition of sea water, the asters reappear, though not attaining full development, and progressive nuclear division may occur without cytoplasmic cleavage. In this case the egg may give rise to a syncytium, containing from four to sixty-four or more nuclei, which migrate towards the periphery so as to take up nearly the same position as they would have had in a segmented blastula. This phenomenon strikingly recalls that which normally occurs in the cleavage of many arthropod and some coelenterate eggs. At each nuclear division an attempt is made at a corresponding cytoplasmic division, but this is usually unsuccessful; or, in case division occurs, the cells subsequently fuse together to repeat the attempt at the next nuclear division. This, again, is closely similar to the ineffective early attempts at cleavage in such eggs as those of *Renilla*. If the eggs be replaced in sea water when the process is not too far advanced (4–32 nuclei), cleavage may occur of a multiple type almost exactly like that occurring in *Renilla*, and a normal blastula may arise; but the cleavage is often irregular or incomplete.

These observations support the conclusion indicated in the preceding paper, that the astral rays are not fixed and permanent structures, but an expression of a form of cytoplasmic activity, partly in the nature of protoplasmic currents, that may be inhibited by temporary paralysis of the cytoplasm. They indicate also that the astral rays are connected with cytoplasmic rather than with nuclear division, and support the interpretation, offered by the author many years ago, of the variations of cleavage observed in *Renilla*.

B.

If *Toxopneustes* eggs be placed in 2.5% ether solution one minute after fertilization, formation of the sperm-aster is completely suppressed. The sperm nucleus, however, slowly moves inwards and gradually enlarges, becoming finally (1–2 hours) as large as the egg nucleus and indistinguishable from it. In some cases, though this is not very common, the two nuclei approach and finally completely fuse to form a typical cleavage nucleus. If in the earlier stages of the process (before union of the germ nuclei and while the sperm nucleus is still not more than two-thirds the diameter of the egg nucleus) the eggs be replaced in pure sea water, the sperm-aster is rapidly developed, centering in a point at one side of the sperm nucleus, and development may proceed normally; but this result was never obtained after the germ nuclei had united, probably because the action of the ether had been too prolonged. In a few cases, after replacing the eggs in sea water, the sperm-aster was observed to divide and form an amphiaster before union of the germ nuclei. In this case the sperm nucleus at the time of union had assumed the vesicular form, though still somewhat smaller than the egg nucleus. One such case was followed out and found to give rise to a normal larva. In such cases the effect of the ether has been to transform the type of fertilization from that characteristic of the sea urchins into that observed in starfishes, or in many worms and mollusks, where an amphiaster is formed before union of the germ nuclei and the latter are approximately equal at the time of union.

The foregoing facts show, in general accordance with the early work of O. and R. Hertwig, that growth of the sperm nucleus and approach and fusion of the germ nuclei may take place quite independently of the sperm-aster; further, that approach of the nuclei is probably not a simple chemotactic phenomenon, since it is very greatly delayed by etherization of the egg.

C.

In some of the etherized eggs, after replacement in sea water, the nucleus failed to divide at the first cleavage, the whole of the chromatin passing to one pole and re-forming as a single nucleus. Such eggs divide into a nucleated and a non-nucleated half, the latter containing only an aster, as in the case of some of the non-nucleated egg fragments fertilized by a single spermatozoön observed by Boveri. In such cases the asters in both halves multiply progressively at the same rate, but complete division occurs in only the nucleated half. In the non-nucleated half, however, each aster becomes surrounded by a deep constriction which afterwards fades out. This result stands intermediate between those of Boveri and Ziegler. As in the case of the magnesium eggs, an aster unaccompanied by nuclear material forms a division center of the surrounding cytoplasmic area, but is here apparently unable to effect complete division in the absence of chromosomes.

XIV. EXPERIMENTS UPON THE INFLUENCE OF THE SEXUAL CELLS UPON THE SOMATIC CELLS.

GEORGE WILTON FIELD.

1. *Of the sexual cells of one individual upon the somatic cells of another individual.*

This is generally held to be a phase of Telegony. Several instances have been cited by Gadow, Nathusius, and Bulman to show that the spermatozoa affect the somatic cells which secrete the eggshell. Thus, the shell of eggs of fowls which normally lay brown eggs are said to become lighter in color when the birds are mated with a male of a breed which lays white eggs; and conversely, white eggs become brownish, if the birds are mated with a male of a breed which lays brown eggs.

Our experiments were carried on at the Rhode Island Agricultural Experiment Station upon Leghorn pullets (white eggs) mated to a Light Brahma cock, and upon Light Brahma pullets (brown eggs) mated to a Leghorn cock. By means of trap nests the series of eggs laid by each individual was followed; no departure from the ordinary normal color of the eggshell could be observed.

2. *Of the sexual cells upon the somatic cells of the same individual.*

In addition to observations upon the changes in the secondary sexual characteristics induced by castration, the effect of absorption of testicular material through the peritoneum was the end in view.

Upon castration the testes of the cockerel were returned free into the abdominal cavity. After six weeks a living ripe testis, one inch long, was introduced into the abdominal cavity through an incision anterior to the last pair of ribs. The experiment was conducted on a scale too small to absolutely determine how far the results were due to direct regeneration of the testes, and how far to absorption of the introduced testicular material. Two points, however, were demonstrated: (1) that the testes do regenerate; (2) that the introduced testicular material was absorbed. In one case it seemed clear that the secondary sexual characteristics developed solely as the effect of the absorption of the introduced testicular material, without regeneration of the testes. In another individual similar conditions were strongly indicated, but were obscured by a pathological growth.

XV. THE MORPHOLOGICAL PHENOMENA INVOLVED IN THE CHEMICAL PRODUCTION OF PARTHENOGENESIS IN SEA URCHINS.

EDMUND B. WILSON.

In accordance with Loeb's important discoveries on *Arbacia*, unfertilized eggs of *Toxopneustes*, when treated by the magnesium chloride method, may segment and give rise to free-swimming blastulas, gastrulas, and Plutei. There is always a considerable proportion of abortive and monstrous forms, and none of the stages are exactly like those arising from fertilized eggs, though often closely similar to them. The Plutei possess, however, the characteristic arms, pigment, skeleton, and divisions of the gut. That these eggs have not been accidentally fertilized is proved by the fact, demonstrated to the society by an exhibition of sections, that during cleavage they show but half the usual number of chromosomes, namely, eighteen instead of thirty-six. The same conclusion is reached by the study of the other internal phenomena, which differ in a characteristic way from those occurring in normal fertilization, though showing an interesting parallel to them.

The eggs, even of the same individual, show a very high degree of variability in their response to the solution. Great numbers of incomplete or abnormal forms of mitosis occur. The most interesting of these are cases in which the nucleus becomes the center of formation of a single aster (monaster), which never resolves itself into an amphiaster but nevertheless passes through periodic changes parallel to those occurring in complete mitosis. Thus, such an aster may appear, nearly disappear, and reappear as many as six times in succession, the nucleus simultaneously disappearing and re-forming. In such cases the chromosomes divide, probably at each disappearance of the nucleus, and may thus become very numerous, without division of the nucleus or of the cell body. In other forms of incomplete mitosis the single aster may give rise to an amphiaster, but the nucleus fails to divide.

In all the eggs capable of development, the initial change is an irregularity in the cytoplasmic meshwork, followed by the appearance of a *primary radiation* centering in the nucleus, the gradual formation of a perinuclear clear zone of hyaloplasm, and the growth of the nucleus. In many of the eggs a number of separate asters (cytasters, equivalent to the "artificial astrospheres" of Morgan), having no direct relation to the nucleus, are formed in the cytoplasm in addition to the primary radiation. At the centers of these asters hyaloplasm likewise accumulates. Growth of the nucleus is followed by disappearance of the nuclear membrane, the rays of both the primary radiation and of the cytasters meanwhile becoming much reduced and in some cases nearly disappearing. After a short pause this is followed by a redevelopment of the rays, and in typical cases the nuclear area (the center of the former primary radiation) has now formed two centers of radiation, producing a typical amphiaster. When no cytasters are present (a relatively rare case) cleavage may proceed nearly as in normally fertilized eggs, but in many cases complete cytoplasmic division does not occur until after two or more nuclear divisions. Thus arise some of the forms of multiple cleavage observed by Morgan and Loeb. If cytasters be present, one or more of them may participate in the nuclear division, thus forming triasters, tetrasters, etc.; but such eggs are probably incapable of producing an embryo. When the cytasters do not establish a connection with the chromosomes, they nevertheless form, in many cases, ineffective centers of cytoplasmic division, *i.e.*, cleavage furrows appear between them but afterwards fade out. Apparently strong evidence was, however, obtained that in some cases complete division may occur around asters unconnected with nuclear material. In any case the cytasters persist for some time and may progressively multiply by division. The first division actually observed takes place nearly synchronously with that of the cleavage asters, at a time when the daughter-nuclei have been formed and are rapidly enlarging. Division of the asters is in both cases preceded by a great reduction of the astral rays, leaving the clear hyaloplasmic central mass surrounded by only short irregular rays, and at the same time the aster migrates out towards the periphery of the egg. The mass then draws apart into two, and recrudescence of the rays from the two centers ensues. A discrepancy, not yet fully cleared up, lies in the fact that, although the cytasters divide synchronously with the cleavage asters, they have not been observed in the living eggs to divide at the time the dicentric nuclear figure is first formed; but the study of sections indicates that this is probably owing to a gap in the observations. The cytasters ultimately disappear.

Asters are formed also in *enucleated fragments*, obtained by shaking the unfertilized eggs into pieces, and such asters may also progressively divide, though no case was observed of cleavage, or even an attempt at cleavage, in such fragments. Sections show that all the asters, whether cytasters or nuclear asters, or those formed in enucleated fragments, contain centrosomes which have the typical staining capacity and granular structure observed in normally fertilized eggs. In the cytasters, however, they are usually smaller than in the nuclear asters, and those in the enucleated fragments are smaller still and often not demonstrable.

The observations indicate that the astral rays, whatever be their other functions, are in part an expression of centripetal currents of hyaloplasm (continuous or interalveolar substance), which lead to the formation of the perinuclear hyaloplasmic zone, and of the clear centers of the cytasters — a conclusion essentially in agreement with the early views of Fol. They show further that the asters (centrosomes) must be regarded as centers of cytoplasmic division, though not ordinarily effective unless connected with nuclear material. They seem to leave no doubt, finally, of the formation *de novo* of functional asters and centrosomes, capable of division, and show that such formation may be entirely independent of the nucleus.

XVI. METAMORPHOSIS IN THE HERMIT-CRAB.

M. T. THOMPSON.

In *Eupagurus longicarpus* only the first six larval stages are distinct: the four zoëas, the important glaucothoë, and the first of the adolescent stages. In the zoëas and the early part of the glaucothoë stage, the "livers" or midgut diverticula are cephalic and thoracic. There are two pairs of these; a pair of *Lesser Lobes* opening dorsally into the stomach, and a pair of somewhat four-lobed *Greater Lobes* opening laterally into the stomach.

During the glaucothoë stage, however, three of the divisions of the greater lobes become atrophied. The fourth or posterior division, at about the time of the second or third day in the shell, grows back into the abdomen. But the lobe of the right side of the body crosses under the intestine to the left, so that both lobes lie on the left of the intestine, which is thrown to the right. At this time the bladders of the Green Glands also migrate into the abdomen. Then the appendages which will be lost become atrophied, and the body musculature alters to the adult type. So the glaucothoë, which was at first Macruran, attains the Eupagurid structural plan before the moult to the sixth stage occurs.

The duration of the glaucothoë stage is dependent on the time of entering the shell. Specimens which take the shell within the first twenty-four hours after the moult from the fourth zoëa, spend only *four* or more often *five* days in the stage. A delay of four days before the shell is taken prolongs this period to *six* or, in a few cases, to *seven* days. In fifty glaucothoë kept without any shells, some remained in the stage the minimum *four* days, but the majority remained *six* and *seven* days, and one remained *eight* days.

The sixth and following stages introduce no important changes in structure, except the branching of the liver. In this branching, however, the majority of the diverticula of the right lobe go to the right under the intestine, so that this lobe, *in the adult*, apparently lies on this, its own side, of the intestine. The lesser lobes branch later and finally form a small tuft of tubules on each side of the stomach.

XVII. ESSENTIAL FACTORS IN THE REGENERATION OF PLANARIA MACULATA.

CHARLES RUSSELL BARDEEN.

Regeneration of a new whole individual from a small piece of a parent individual depends in *Planaria maculata* upon the presence in the piece of a part of the central nervous system and a part of the intestinal system. A small piece containing these parts will regenerate from them new typical intestinal and nervous systems. At the same time the parenchyma in the vicinity of the cut surface becomes increased in amount, and is symmetrically differentiated in relation to the new intestinal system. A head may thus be formed anterior to a new axial gut, and lateral and tail areas may be restored. Polarity of the piece is determined by the central nervous apparatus which it contains.

A new pharynx is formed just posterior to the point where intestinal contents collect when the whole piece contracts.

The pharynx may be formed in a region of the piece at some distance from the cut surface. Head, lateral, and tail areas are differentiated only at a cut surface.

The reproductive organs are not regenerated. Instead, they disappear from a small piece isolated from a sexually mature worm. The tail cut from a planarian in which the reproductive organs are developing will give rise to regenerative forces which overpower the forces giving rise to the sexual organs. Regeneration is equally rapid in sexually mature and in sexually immature worms.

In regeneration in this animal the tissues seem to be specific, except that the new musculature probably comes from parenchyma cells.

A full account of the Physiology of Regeneration in these animals is given in the *American Journal of Physiology*, Vol. V (1901), p. 1.

XVIII. THE HISTOGENESIS OF THE PERIPHERAL NERVOUS SYSTEM IN SALMO SALAR.[1]

ROSS GRANVILLE HARRISON.

CELLS provided with pseudopodia-like processes wander out singly from the dorsal surface of the medullary cord, and collect together between the myotomes and the cord into small groups, the spinal ganglia. Here the cells remain for some time undifferentiated, but are transformed later into bipolar cells, of which the centripetal processes grow into the side of the medullary cord to form the dorsal roots.

Neuroblasts may be distinguished at an early stage as round or polyhedral cells, lying in the outer zone of the cord. At this period the cord is made up chiefly of epithelial cells, the forerunner of the ependyma. These cells are still undifferentiated, no specialized "*Randschleier*" being present. As the axones grow out from the neuroblasts, they bore their way through the substance of the epithelial cells, which with the continued growth of new fibres become more and more honeycombed. Their outer zone is finally transformed into a fibrous framework, the "*Randschleier*," which accordingly owes its structure to the activity of the growing nerve fibres, and is not pre-formed.

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[1] A full account of this work is published in the *Archiv für mikroskopische Anatomie*, January, 1901.

The dorsal cells or giant cells of Rohon arise in the dorsolateral portion of the cord next the outer limiting membrane. They elongate, and for the most part each cell gives rise to an ascending and a descending nerve fibre forming the beginning of the dorsal funiculi. Gradually the cells leave their original position and wander to the dorsal mid-line of the cord. Through this movement the cells become unipolar, remaining connected with their fibres by a slender process, which divides in T-fashion at the point where the longitudinal fibres begin. A large number of the cells form peripheral nerves also, which are segmentally arranged. The dorsal cells are homologous with the "*Hinterzellen*" found in *Petromyzon*, and with the bipolar cells of medium size in the cord of *Amphioxus*. They are to be regarded as a primitive type of sensory cell identical in function with the spinal ganglion cells, with which they are genetically related.

XIX. THE SPERMATIC AND MESENTERIC ARTERIES OF DIDELPHYS VIRGINIANA (KERR, LINN.).

C. F. W. McCLURE.

In mammals other than marsupials the anterior mesenteric artery supplies the small intestine and the proximal end of the large intestine. The posterior mesenteric artery is given off from the posterior division of the abdominal aorta, and supplies the large intestine. In a large number of mammals the internal spermatic arteries are given off from the aorta about midway between the renal and posterior mesenteric arteries.

In *Didelphys* and other marsupials, so far as known to the writer, the anterior mesenteric artery supplies both the small and large intestines. In *Didelphys* and other marsupials the posterior mesenteric artery *is not present.* Also in *Didelphys* and other marsupials the internal spermatic arteries are given off from the posterior division of the aorta, and at a point which coincides with the point of origin of the posterior mesenteric artery in other mammals.

In an adult *Didelphys* killed during the breeding season the writer found present two pairs of functional internal spermatic arteries. The anterior pair was given off from the aorta about midway between the renal and posterior pair of internal spermatic arteries. The posterior pair, the so-called internal spermatic arteries of marsupials, was given off from the aorta in the usual manner, as mentioned above. More recently the writer has found another adult female *Didelphys*, in which, in addition to two pairs of internal spermatic arteries, a large *posterior mesenteric artery was present.*

In this individual the posterior mesenteric artery arose from the aorta as a single vessel, and at a point which coincided with the origin of this vessel in other mammals. On arising from the aorta the vessel passed ventrad through a foramen in the vena cava, and was distributed to the large intestine. The anterior mesenteric artery in this individual supplied the small intestine and the proximal portion of the large intestine.

The relations of the spermatic arteries were as follows:

The anterior pair of internal spermatic arteries arose from the aorta, and was distributed to the ovaries as in the above-mentioned case. These arteries appear to be the homologues of those spermatic arteries which in many other mammals arise from the aorta about midway between the renal and posterior mesenteric arteries. The posterior pair of internal spermatic arteries in this opossum were *branches of the posterior mesenteric artery*, and were given off from this vessel near its point of origin at the aorta.

It appears to the writer as though in the marsupials, as the result of an arrested development of the original internal spermatic and the posterior mesenteric arteries, a new collateral circulation has been established to the genital organs and large intestine. The collateral circulation to the large intestine has apparently been established through the anterior mesenteric artery; that to the ovaries, through vessels which may have been formed as the result of a modification of the posterior mesenteric artery.

XX. SOME FACTS CONCERNING REGENERATION AND REGULATION IN RENILLA.

H. B. TORREY.

During the past summer experiments were carried on at Beaufort, North Carolina, preliminary to a more complete investigation of the processes of regeneration and regulation in *Renilla*. It was hoped that *Renilla*, being a polymorphic colonial form, — an *aggregate of polyps and zoöids*, — would behave like a simple metazoan individual, and at the same time offer surer landmarks, during regulative processes, than a metazoan individual — *an aggregate of cells;* for changes in polyps as a whole may be perceived more clearly than changes in their component cells.

The results may be summarized as follows:

1. *Renilla* colonies may regenerate lost parts readily.

2. They exhibit a strong polarity. When a peduncle is removed by a transverse cut an axial polyp is never regenerated in its place, and *vice versa*.

3. There is an anterior limit beyond which anterior pieces do not regenerate posteriorly, and a posterior limit beyond which posterior pieces do not regenerate anteriorly. These correspond to the limits of the budding zone.

4. The colonies regulate themselves in a plastic fashion when cut in certain ways, obliquely, for instance. It is thus possible to obtain two new colonies, one of which retains the original peduncle with a lateral polyp displaced into the position formerly occupied by the axial polyp. Whether or not the colony develops symmetrically around this new axis is not known.

If the oblique cut makes with the colonial axis an angle larger than forty-five degrees, there is no displacement of the lateral polyp, the extirpated axial polyp regenerating as though it alone had been removed by a transverse cut.

5. When a lateral group of polyps is removed by a longitudinal cut, it regenerates a new peduncle approximately at a right angle to the cut surface, and approximately in the axis of the chief lateral polyp of the group. The future of such pieces is unknown. This is a case of heteromorphosis.

XXI. SOME POINTS IN THE BRAIN OF LOWER VERTEBRATES.

J. B. JOHNSTON.

The central olfactory apparatus of *Petromyzon* presents, in all important features, an extraordinary resemblance to that of *Acipenser*. In *Petromyzon*, on account of the great buccal apparatus, there has occurred a sort of telescoping of the olfactory lobes and areas upon the striatum and thalamus as fixed points. The so-called cortex, described by Friedrich Mayer, is nothing else than the epistriatum.

The cells of the olfactory lobe present more primitive characters in *Petromyzon* than in *Acipenser*. The mitral cells are only slightly differentiated, while the stellate and other cells are very numerous and send their neurites, along with those of the mitral cells, to the olfactory nuclei of the fore-brain. Similar categories of cells have been described in Amphibia (P. R. Cajal) and reptiles (Edinger's "Lobuscortex"), although differently interpreted. The numerous, slightly differentiated cells in the olfactory lobe of *Petromyzon* and *Acipenser* represent the material from which the highly differentiated elements of the olfactory lobe of higher vertebrates have been developed.

Several authors have pointed out the close connection between the cerebellum and acusticum in fishes. The study of the minute structure shows that the cerebellum is derived directly from the front end of the acusticum. Evidence for this from *Acipenser:*

a. Gross continuity of cerebellum and acusticum.

b. The root fibres of the fifth, eighth, and lateral line nerves enter and end in both.

c. The several categories of types of cells in the cerebellum — Purkinje cells, granules, and cells of the second type — are strictly homologous with similar cells found in the acusticum.

d. The development of the Purkinje cells in the acusticum from the typical large cells of that nucleus is in actual progress and may be studied in all its stages.

Additional evidence in *Petromyzon:*

a. The Purkinje cells in the cerebellum are not well developed, and their neurites run to the base of the mid-brain, possibly having the same destination as the internal arcuate fibres from the acusticum.

b. The *tractus tecto-cerebellaris* seems to be absent and the *tractus lobo-cerebellaris* is small.

c. The cerebellum is little more than a dorsal arch and commissure from the front end of the acusticum.

XXII. ASEXUAL REPRODUCTION OF PLANARIA MACULATA.

WINTERTON C. CURTIS.

The fission of *Planaria maculata,* while it does not differ essentially from the type found in other planarians where fission occurs, is of just the right sort to complete a very interesting series and connect the fission of land planarians, which is hardly more than a fragmentation, with the fission of *Planaria fissipara,* in which the organs are completely formed before the new individuals separate. This series is as follows: (1) Land planarians, in which pieces of varying lengths are pinched off from the posterior end; (2) *Planaria maculata,* which divides always at the same place behind the pharynx, with no preformation of organs; (3) *Planaria subtentacula*

(Zacharias, *Zeit. f. Wiss. Zool.*, 1886, Bd. XLIII, pp. 271–275), where there is some rearrangement of the gut and the pharynx is partly developed before separation; (4) *Planaria fissipara* (Kennel, *Zool. Jahrb.*, Abth. f. Anat. u. Ontog., 1888, Bd. III, pp. 447–486), in which a complete worm is developed out of the posterior third of a large specimen and both reach normal proportions before separation. In the last three cases the division occurs at a corresponding place.

The division in *Planaria maculata* seems to be brought about by a contraction of the circular muscles, which pinches the individual in two a short distance behind the pharynx. The cut ends of either piece are as though they had been produced by a knife-cut, and examination of sections shows that the parenchyma at the scar is actually naked. There is nothing like a furrow on the outside previous to the division, which, nevertheless, is a regular and normal reproductive process and not induced by any ordinary irritation or mutilation of the animals. The large number of pieces in various stages of regeneration, found in collecting, is sufficient evidence of the occurrence of the fission under natural conditions.

Worms will not divide in the laboratory to any considerable extent unless well fed. If the water has become foul and is replaced by fresh, a considerable number of specimens will usually be found divided within the next twelve hours. The division usually occurs at night, whether the dishes are shaded or not. The morpholaxis of the head and tail pieces resulting from a division is rapid. Tail pieces may reach almost the normal proportions and re-divide in from five to six days if well fed, heads in not less than ten days. There is no regular interval between the divisions.

In certain localities this species does not possess reproductive organs at any time during the year, but has during the summer months an active period of asexual multiplication. In other localities the worms develop these organs in the fall and lay eggs in the spring; and although all the specimens are without these organs at the end of the summer, asexual reproduction has never been observed. In another sexual locality the worms, when they are without reproductive organs at the end of the summer, do reproduce asexually to a considerable extent. Later this ceases and the reproductive organs develop. These statements are based upon observations extending over from two to three years.

A possible explanation is that the asexual reproduction may be substituted for the sexual in certain localities during considerable periods, but further data are necessary to confirm this.

XXIII. VARIATION AMONG HYDROMEDUSAE.

CHARLES W. HARGITT.

OBSERVATIONS upon variations among the Hydromedusae seem to have been of comparatively limited extent. References to the subject are to be found in the writings of Ehrenberg, Forbes, Agassiz, Hincks, Romanes, and later by Agassiz and Woodworth, but except in the last-named paper they are rather incidental and fragmentary.

Of my own observations only the barest abstract and summary can be undertaken in this connection.

Among the genera studied the principal have been as follows: *Pennaria*, *Eucope*, *Obelia*, *Margelis*, *Gonionemus*, *Nemopsis*,, *Rhegmatodes*.

The principal organs examined were: (1) The Chymiferous Canals, (2) Tentacles, (3) Gonads, (4) Otocysts. Among these the greatest range of variation was noted in the tentacles, as might naturally be expected, in some cases reaching as high as 90 per cent. In the forking and doubling of tentacles there was least, rarely exceeding 5 per cent, and indeed seldom reaching that ratio; in *Gonionemus* 3 per cent.

In the looping, branching, and anastomosing of chymiferous canals there was great variation in different genera, in some being almost *nil*, while in others (*Eucope* and *Gonionemus*) varying from 5 to 10 per cent.

Considerable variation was found in the gonads, though less than in the other organs already noted, varying in different genera from 2 to 5 per cent. While considerable variation was evident in the number, arrangement and correlation of the otocysts, no attempt has been made to ascertain the exact ratio, owing to the difficulty attending this determination in preserved specimens.

The following summary will express in a general way some of the more evident conclusions reached:

1. Variation among Hydromedusae is of wider extent than had been suspected.
2. It is much greater in some genera than in others.
3. It seems to be much less symmetrical and correlated than among Scyphomedusae.
4. Many phases of variation appear to be wholly devoid of correlative and adaptive aspects.

XXIV. EXPERIMENTS ON MODIFYING THE NORMAL PROPORTION OF THE SEXES IN THE DOMESTIC FOWL.

GEORGE WILTON FIELD.

This is a brief report on a series of experimental attempts to ascertain the factors which determine sex.

The normal proportion given by Darwin from observation of 1001 chicks during eight years was 94.7 males to every 100 females. From 2105 chicks during two years, we found the proportion to be 80.6 males to every 100 females.

These figures lead us to query whether the normal proportion may not have changed during the past forty years as a result of the breeders' desire to produce a larger proportion of females.

In the experiments attempts were made to isolate the factors so that the effects of each could be observed:

1. Absolute age of parents:
    - 9 young females mated to male of same age.
    - 9 old " " " " " " "
2. Relative age of parents:
    - 9 old females mated to young male.
    - 9 young " " " old "

The proportion practically coincided with the normal except in the case of young females mated to old male, where a slight increase in the number of males appears.

3. Malnutrition (a result of feeding to one-half usual amount): a very marked increase in number of males; rate of 176.6 males to every 100 females.
4. Scarcity of males, *i.e.*, polygamy: 20 females mated to 1 male, a marked increase in number of males; 139.6 males to every 100 females.
5. Conditions connected with time of year: 650 chicks hatched between March 15 and May 15 gave a smaller proportion of males, — 73.9 males to every 100 females; while 471 chicks hatched between May 15 and July 15 gave a larger proportion of males, — 88.4 males to every 100 females.

It is to be understood that these figures are for a relatively small number of cases; it is hoped to extend them to at least 10,000 individuals.

XXV. NOTES ON VARIATION IN THE SHELLS OF PURPURA LAPILLUS.

R. P. BIGELOW AND H. S. CONANT.

Purpura lapillus is a species that presents great variations in its diagnostic characters. It was thought, therefore, that a study of its variations by statistical methods might be of value in defining more exactly the limits of the species, and might also bring to light facts of general biological interest. Collections were made at Prince's Cove and on the mainland opposite Clark's Ledge, Eastport, at Kennebunk Beach, at Bass Rocks, Gloucester, and at Newport. The sexes were separated for each locality, and the following characters were measured:

(1) Angle of the apex and nuclear whorls, or nuclear angle; (2) angle of the apex and the last whorl, or adult angle; (3) total length; and (4) length of spire from the apex to the posterior margin of the opening of the shell. Record was made also of the presence of (5) imbrications, (6) sutures, (7) ribs, (8) teeth, (9) of the curvature of the columella, and (10) of the weight. Perhaps the most obvious variations are in the color and the thickness of the shell, but no satisfactory method was

found for measuring these quantities. A special instrument is being constructed which, it is hoped, will overcome the difficulty in regard to thickness.

A preliminary study of the shells from Eastport and Gloucester shows that for characters (1) to (4) the variations may be represented by curves that are approximately normal. The curves for the two stations at Eastport fit together pretty closely; while they differ distinctly from the curves for the Gloucester specimens, the difference of the means being greater than the standard deviation for each locality. In each case the female shells showed, on the average, a wider angle and a shorter spire expressed in per cent of total length, than the corresponding males, and the same is true for the Gloucester shells as a whole compared with the Eastport shells.

As a measure of variability the coefficient of variation $\left(cv. = 100\frac{\sigma}{m}\right)$ gives contradictory results and appears not to be applicable to measurements expressed in degrees of a circle. Judging from the standard deviations, the shells from Gloucester are somewhat more variable than those from Eastport. The relative variability of the males and females differs for the different characters, and for the same characters in different localities. In general, the females appear to be slightly more variable than the males.

XXVI. VARIATION AND ELIMINATION IN PHILOSAMIA CYNTHIA.

HENRY E. CRAMPTON.

SOME of the results were presented of a statistical study in the case of a large Saturnid moth, *P. cynthia*, of the variability of eliminated and surviving pupae and imagines. From a lot of nearly 1100 cocoons, only 310 living pupae were obtained; 632 contained dead pupae; while the remainder were shriveled or otherwise abnormal larvae or pupae. The living pupae were compared with an equal number of dead pupae with reference to certain body characters: length, length of bust (to fifth abdominal segment), width and depth of bust, frontal stature of bust (ratio of middle to length), and sagittal stature of bust (ratio of depth to length). In addition the length, width, and stature of a typical organ, the left antenna, were determined. From a tabulation of certain bases of comparison — mean, standard deviation, coefficient of variability — it appeared: (1) That eliminated male pupae were on the whole more variable than the surviving males, and that the surviving females were far less variable than the dead ones. (2) Only 180 of the 310 living pupae produced perfect moths. The perfect male moths were from pupae which were far less variable than the others. This condition was reversed in the case of the females, yet the surviving females, though more variable than the eliminated ones, were not as variable as the eliminated female pupae. (3) The males of all groups were more variable than the females.

XXVII. THE ORIGIN OF TENTACLES IN GONIONEMUS.

H. F. PERKINS.

Some interesting data have been secured from the study of the origin of tentacles in *Gonionemus*, a common Woods Holl hydromedusa. Specimens $\frac{1}{2}$ mm. in diameter having from 8 to 16 tentacles are found in early summer, and in examining these it was seen that there existed a definite relative position of the tentacles and sense organs.

Two pairs of tentacles, the radial ones, are of equal size. The other tentacles and sense organs are regularly graded from large to small, so that it is possible to determine their order of origin.

Looking at the marginal ring from below, in a normal medusa, each newly formed tentacle is seen to lie next to a sense organ and to precede it, as the hands of a watch move. Fig. 1 shows a typical 8-tentacled medusa. Tentacles I and II are radial in position; III follows I in the direction of the hands of a watch; IV follows II, and the two pairs of sense organs, 1 and 2, lie as if the cells that were to form them had been crowded along to the right by the newly formed tentacles.

In an older specimen (Fig. 2) the successive pairs of tentacles and sense organs have arisen in corresponding positions, as is indicated by the numbers on the diagram. The origin

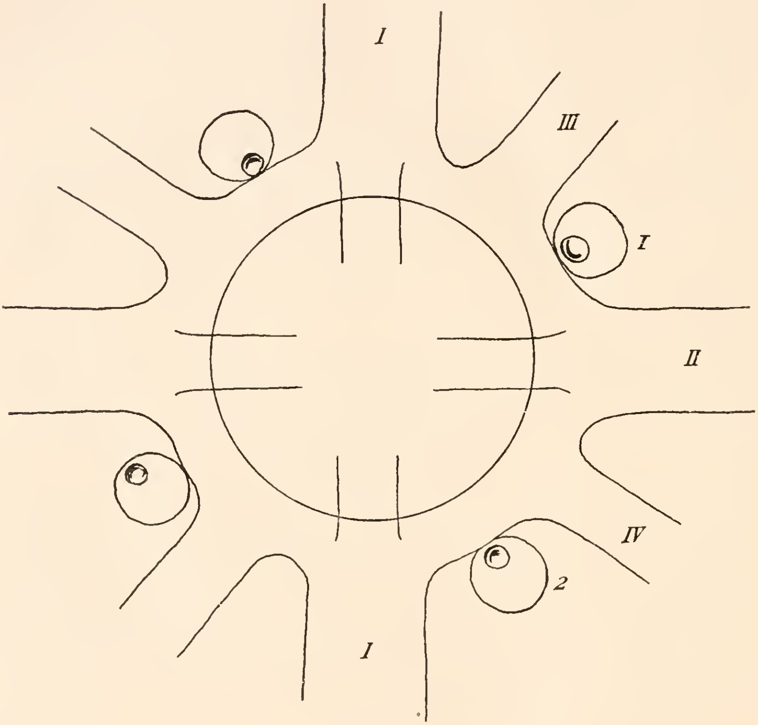

FIG. 1.

of tentacles and sense organs would seem then to be governed by an attempt at radial symmetry which is constantly interfered with by this sequence of formation from left to right, along the bell-margin.

In full-grown medusae there appears a striking conformity to this rule, with fewer exceptions than would be expected from the frequency of other variations in all parts of the creature.

The comparison of other and allied forms, with this rule in mind, may bring to light some interesting facts bearing on the correspondence of parts and on radial and bilateral symmetry.

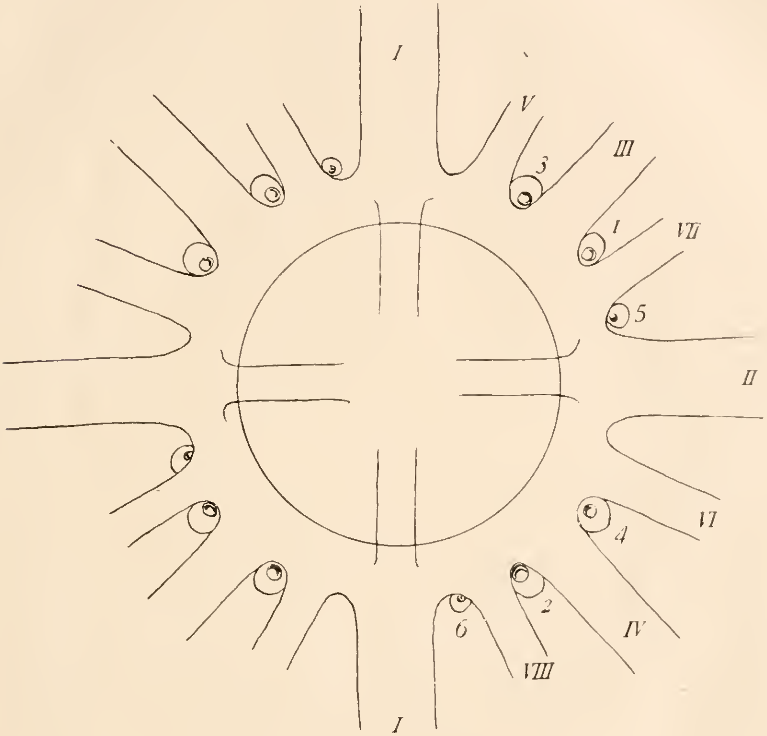
I
V
3
III
1
VII
5
II
VI
4
2
IV
6
VIII
I

Fig. 2

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